89-97

STARCH ENCAPSULATION

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to provisional patent application serial No. 60/026,855 filed September 30, 1996. Said provisional application is incorporated herein by reference to the extent not inconsistent herewith.

BACKGROUND OF THE INVENTION

Polysaccharide Enzymes

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Both prokaryotic and eukaryotic cells use polysaccharide enzymes as a storage reserve. In the prokaryotic cell the primary reserve polysaccharide is glycogen. Although glycogen is similar to the starch found in most vascular plants it exhibits different chain lengths and degrees of polymerization. In many plants, starch is used as the primary reserve polysaccharide. Starch is stored in the various tissues of the starch bearing plant. Starch is made of two components in most instances; one is amylose and one is amylopectin. Amylose is formed as linear glucans and amylopectin is formed as branched chains of glucans. Typical starch has a ratio of 25% amylose to 75% amylopectin. Variations in the amylose to amylopectin ratio in a plant can effect the properties of the starch. Additionally starches from different plants often have different properties. Maize starch and potato starch appear to differ due to the presence or absence of phosphate groups. Certain plants' starch properties differ because of mutations that have been introduced into the plant genome. Mutant starches are well known in maize, rice and peas and the like.

The changes in starch branching or in the ratios of the starch components result in different starch characteristic. One characteristic of starch is the formation of starch granules which are formed particularly in leaves, roots, tubers and seeds. These granules are formed during the starch synthesis process. Certain synthases of starch, particularly

granule-bound starch synthase, soluble starch synthases and branching enzymes are proteins that are "encapsulated" within the starch granule when it is formed.

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The use of cDNA clones of animal and bacterial glycogen synthases are described in International patent application publication number GB92/01881. The nucleotide and amino acid sequences of glycogen synthase are known from the literature. For example, the nucleotide sequence for the *E. coli* glgA gene encoding glycogen synthase can be retrieved from the GenBank/EMBL (SWISSPROT) database, accession number J02616 (Kumar et al., 1986, J. Biol. Chem., 261:16256-16259). *E. coli* glycogen biosynthetic enzyme structural genes were also cloned by Okita et al. (1981, J. Biol. Chem., 256(13):6944-6952). The glycogen synthase glgA structural gene was cloned from *Salmonella typhimurium* LT2 by Leung et al. (1987, J. Bacteriol., 169(9):4349-4354). The sequences of glycogen synthase from rabbit skeletal muscle (Zhang et al., 1989, FASEB J., 3:2532-2536) and human muscle (Browner et al., 1989, Proc. Natl. Acad. Sci., 86:1443-1447) are also known.

The use of cDNA clones of plant soluble starch synthases has been reported. The amino acid sequences of pea soluble starch synthase isoforms I and II were published by Dry et al. (1991, Plant Journal, 2:193202). The amino acid sequence of rice soluble starch synthase was described by Baba et al. (1993, Plant Physiology,). This last sequence (rice SSTS) incorrectly cites the N-terminal sequence and hence is misleading. Presumably this is because of some extraction error involving a protease degradation or other inherent instability in the extracted enzyme. The correct N-terminal sequence (starting with AELSR) is present in what they refer to as the transit peptide sequence of the rice SSTS.

The sequence of maize branching enzyme I was investigated by Baba et al., 1991, BBRC, 181:8794. Starch branching enzyme II from maize endosperm was investigated by Fisher and Shrable (1993, Plant Physiol., 102:10451046). The use of cDNA clones of plant, bacterial and animal branching enzymes have been reported. The nucleotide and amino acid sequences for bacterial branching enzymes (BE) are known from the literature. For example, Kiel et al. cloned the branching enzyme gene glgB from *Cyanobacterium synechococcussp* PCC7942 (1989, Gene (Amst), 78(1):918) and from *Bacillus*

stearothermophilus (Kiel et al., 1991, Mol. Gen. Genet., 230(12):136-144). The genes glc3 and ghal of S. cerevisiae are allelic and encode the glycogen branching enzyme (Rowen et al., 1992, Mol. Cell Biol., 12(1):22-29). Matsumomoto et al. investigated glycogen branching enzyme from Neurospora crassa (1990, J. Biochem., 107:118-122). The GenBank/EMBL database also contains sequences for the E. coli glgB gene encoding branching enzyme.

Starch synthase (EC 2.4.1.11) elongates starch molecules and is thought to act on both amylose and amylopectin. Starch synthase (STS) activity can be found associated both with the granule and in the stroma of the plastid. The capacity for starch association of the bound starch synthase enzyme is well known. Various enzymes involved in starch biosynthesis are now known to have differing propensities for binding as described by Mu-Forster et al. (1996, Plant Phys. 111: 821-829). Granule-bound starch synthase (GBSTS) activity is strongly correlated with the product of the waxy gene (Shure et al., 1983, Cell 35: 225-233). The synthesis of amylose in a number of species such as maize, rice and potato has been shown to depend on the expression of this gene (Tsai, 1974, Biochem Gen 11: 83-96; Hovenkamp-Hermelink et al., 1987, Theor. Appl. Gen. 75: 217-221). Visser et al. described the molecular cloning and partial characterization of the gene for granule-bound starch synthase from potato (1989, Plant Sci. 64(2):185192). Visser et al. have also described the inhibition of the expression of the gene for granule-bound starch synthase in potato by antisense constructs (1991, Mol. Gen. Genet. 225(2):289296).

The other STS enzymes have become known as soluble starch synthases, following the pioneering work of Frydman and Cardini (Frydman and Cardini, 1964, Biochem. Biophys. Res. Communications 17: 407-411). Recently, the appropriateness of the term "soluble" has become questionable in light of discoveries that these enzymes are associated with the granule as well as being present in the soluble phase (Denyer et al., 1993, Plant J. 4: 191-198; Denyer et al., 1995, Planta 97: 57-62; Mu-Forster et al., 1996, Plant Physiol. 111: 821-829). It is generally believed that the biosynthesis of amylopectin involves the interaction of soluble starch synthases and starch branching enzymes. Different isoforms of soluble starch synthase have been identified and cloned in pea (Denyer and Smith, 1992, Planta 186: 609-617; Dry et al., 1992, Plant Journal, 2: 193-

202), potato (Edwards et al., 1995, Plant Physiol 112: 89-97; Marshall et al., 1996, Plant Cell 8: 1121-1135) and in rice (Baba et al., 1993, Plant Physiol. 103: 565-573), while barley appears to contain multiple isoforms, some of which are associated with starch branching enzyme (Tyynela and Schulman, 1994, Physiol. Plantarum 89: 835-841). A common characteristic of STS clones is the presence of a KXGGLGDV consensus sequence which is believed to be the ADP-Glc binding site of the enzyme (Furukawa et al., 1990, J Biol Chem 265: 2086-2090; Furukawa et al., 1993, J. Biol. Chem. 268: 23837-23842).

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In maize, two soluble forms of STS, known as isoforms I and II, have been identified (Macdonald and Preiss, 1983, Plant Physiol. 73: 175-178; Boyer and Preiss, 1978, Carb. Res. 61: 321-334; Pollock and Preiss, 1980, Arch Biochem. Biophys. 204: 578-588; Macdonald and Preiss, 1985 Plant Physiol. 78: 849-852; Dang and Boyer, 1988, Phytochemistry 27: 1255-1259; Mu et al., 1994, Plant J. 6: 151-159), but neither of these has been cloned. STSI activity of maize endosperm was recently correlated with a 76-kDa polypeptide found in both soluble and granule-associated fractions (Mu et al., 1994, Plant J. 6: 151-159). The polypeptide identity of STSII remains unknown. STSI and II exhibit different enzymological characteristics. STSI exhibits primer-independent activity whereas STSII requires glycogen primer to catalyze glucosyl transfer. Soluble starch synthases have been reported to have a high flux control coefficient for starch deposition (Jenner et al., 1993, Aust. J. Plant Physiol. 22: 703-709; Keeling et al., 1993, Planta 191: 342-348) and to have unusual kinetic properties at elevated temperatures (Keeling et al., 1995, Aust. J. Plant Physiol. 21 807-827). The respective isoforms in maize exhibit significant differences in both temperature optima and stability.

Plant starch synthase (and *E. coli* glycogen synthase) sequences include the sequence KTGGL which is known to be the ADPG binding domain. The genes for any such starch synthase protein may be used in constructs according to this invention.

Branching enzyme [α1,4Dglucan: α1,4Dglucan 6D(α1,4Dglucano) transferase (E.C. 2.4.1.18)], sometimes called Q-enzyme, converts amylose to amylopectin. A segment of a α1,4Dglucan chain is transferred to a primary hydroxyl group in a similar glucan chain.

Bacterial branching enzyme genes and plant sequences have been reported (rice endosperm: Nakamura et al., 1992, Physiologia Plantarum, 84:329-335 and Nakamura and Yamanouchi, 1992, Plant Physiol., 99:1265-1266; pea: Smith, 1988, Planta, 175:270-279 and Bhattacharyya et al., 1989, J. Cell Biochem., Suppl. 13D:331; maize endosperm: Singh and Preiss, 1985, Plant Physiology, 79:34-40; VosScherperkeuter et al., 1989, Plant Physiology, 90:75-84; potato: Kossmann et al., 1991, Mol. Gen. Genet., 230(12):39-44; cassava: Salehuzzaman and Visser, 1992, Plant Mol Biol, 20:809-819).

In the area of polysaccharide enzymes there are reports of vectors for engineering modification in the starch pathway of plants by use of a number of starch synthesis genes in various plant species. That some of these polysaccharide enzymes bind to cellulose or starch or glycogen is well known. One specific patent example of the use of a polysaccharide enzyme shows the use of glycogen biosynthesis enzymes to modify plant starch. In U.S. patent 5,349,123 to Shewmaker a vector containing DNA to form glycogen biosynthetic enzymes within plant cells is taught. Specifically, this patent refers to the changes in potato starch due to the introduction of these enzymes. Other starch synthesis genes and their use have also been reported.

Hybrid (fusion) Peptides

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Hybrid proteins (also called "fusion proteins") are polypeptide chains that consist of two or more proteins fused together into a single polypeptide. Often one of the proteins is a ligand which binds to a specific receptor cell. Vectors encoding fusion peptides are primarily used to produce foreign proteins through fermentation of microbes. The fusion proteins produced can then be purified by affinity chromatography. The binding portion of one of the polypeptides is used to attach the hybrid polypeptide to an affinity matrix. For example, fusion proteins can be formed with beta galactosidase which can be bound to a column. This method has been used to form viral antigens.

Another use is to recover one of the polypeptides of the hybrid polypeptide.

Chemical and biological methods are known for cleaving the fused peptide. Low pH can be used to cleave the peptides if an acid-labile aspartyl-proline linkage is employed between the peptides and the peptides are not affected by the acid. Hormones have been

cleaved with cyanobromide. Additionally, cleavage by site-specific proteolysis has been reported. Other methods of protein purification such as ion chromatography have been enhanced with the use of polyarginine tails which increase overall basicity of the protein thus enhancing binding to ion exchange columns.

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A number of patents have outlined improvements in methods of making hybrid peptides or specific hybrid peptides targeted for specific uses. US patent 5,635,599 to Pastan et al. outlines an improvement of hybrid proteins. This patent reports a circularly permuted ligand as part of the hybrid peptide. This ligand possesses specificity and good binding affinity. Another improvement in hybrid proteins is reported in U.S. patent 5,648,244 to Kuliopulos. This patent describes a method for producing a hybrid peptide with a carrier peptide. This nucleic acid region, when recognized by a restriction endonuclease, creates a nonpalindromic 3-base overhang. This allows the vector to be cleaved.

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An example of a specifically targeted hybrid protein is reported in U.S. patent 5,643,756. This patent reports a vector for expression of glycosylated proteins in cells. This hybrid protein is adapted for use in proper immunoreactivity of HIV gp120. The isolation of gp120 domains which are highly glycosylated is enhanced by this reported vector.

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U.S. patent 5,202,247 and 5,137,819 discuss hybrid proteins having polysaccharide binding domains and methods and compositions for preparation of hybrid proteins which are capable of binding to a polysaccharide matrix. U.S. patent 5,202,247 specifically teaches a hybrid protein linking a cellulase binding region to a peptide of interest. The patent specifies that the hybrid protein can be purified after expression in a bacterial host by affinity chromatography on cellulose.

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The development of genetic engineering techniques has made it possible to transfer genes from various organisms and plants into other organisms or plants. Although starch has been altered by transformation and mutagenesis in the past there is still a need for further starch modification. To this end vectors that provide for encapsulation of desired

amino acids or peptides within the starch and specifically within the starch granule are desirable. The resultant starch is modified and the tissue from the plant carrying the vector is modified.

SUMMARY OF THE INVENTION

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This invention provides a hybrid polypeptide comprising a starch-encapsulating region (SER) from a starch-binding enzyme fused to a payload polypeptide which is not endogenous to said starch-encapsulating region, i.e. does not naturally occur linked to the starch-encapsulating region. The hybrid polypeptide is useful to make modified starches comprising the payload polypeptide. Such modified starches may be used to provide grain feeds enriched in certain amino acids. Such modified starches are also useful for providing polypeptides such as hormones and other medicaments, e.g. insulin, in a starch-encapsulated form to resist degradation by stomach acids. The hybrid polypeptides are also useful for producing the payload polypeptides in easily-purified form. For example, such hybrid polypeptides produced by bacterial fermentation, or in grains or animals, may be isolated and purified from the modified starches with which they are associated by art-known techniques.

The term "polypeptide" as used herein means a plurality of identical or different amino acids, and also encompasses proteins.

The term "hybrid polypeptide" means a polypeptide composed of peptides or polypeptides from at least two different sources, e.g. a starch-encapsulating region of a starch-binding enzyme, fused to another polypeptide such as a hormone, wherein at least two component parts of the hybrid polypeptide do not occur fused together in nature.

The term "payload polypeptide" means a polypeptide not endogenous to the starchencapsulating region whose expression is desired in association with this region to express a modified starch containing the payload polypeptide. When the payload polypeptide is to be used to enhance the amino acid content of particular amino acids in the modified starch, it preferably consists of not more than three different types of amino acids selected from the group consisting of: Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val.

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When the payload polypeptide is to be used to supply a biologically active polypeptide to either the host organism or another organism, the payload polypeptide may be a biologically active polypeptide such as a hormone, e.g., insulin, a growth factor, e.g. somatotropin, an antibody, enzyme, immunoglobulin, or dye, or may be a biologically active fragment thereof as is known to the art. So long as the polypeptide has biological activity, it does not need to be a naturally-occurring polypeptide, but may be mutated, truncated, or otherwise modified. Such biologically active polypeptides may be modified polypeptides, containing only biologically-active portions of biologically-active polypeptides. They may also be amino acid sequences homologous to naturally-occurring biologically-active amino acid sequences (preferably at least about 75% homologous) which retain biological activity.

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The starch-encapsulating region of the hybrid polypeptide may be a starch-encapsulating region of any starch-binding enzyme known to the art, e.g. an enzyme selected from the group consisting of soluble starch synthase I, soluble starch synthase II, soluble starch synthase III, granule-bound starch synthase, branching enzyme I, branching enzyme IIa, branching enzyme IIBb and glucoamylase polypeptides.

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When the hybrid polypeptide is to be used to produce payload polypeptide in pure or partially purified form, the hybrid polypeptide preferably comprises a cleavage site between the starch-encapsulating region and the payload polypeptide. The method of isolating the purified payload polypeptide then includes the step of contacting the hybrid polypeptide with a cleaving agent specific for that cleavage site.

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This invention also provides recombinant nucleic acid (RNA or DNA) molecules encoding the hybrid polypeptides. Such recombinant nucleic acid molecules preferably comprise control sequences adapted for expression of the hybrid polypeptide in the

selected host. The term "control sequences" includes promoters, introns, preferred codon sequences for the particular host organism, and other sequences known to the art to affect expression of DNA or RNA in particular hosts. The nucleic acid sequences encoding the starch-encapsulating region and the payload polypeptide may be naturally-occurring nucleic acid sequences, or biologically-active fragments thereof, or may be biologically-active sequences homologous to such sequences, preferably at least about 75% homologous to such sequences.

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Host organisms include bacteria, plants, and animals. Preferred hosts are plants. Both monocotyledonous plants (monocots) and dicotyledonous plants (dicots) are useful hosts for expressing the hybrid polypeptides of this invention.

This invention also provides expression vectors comprising the nucleic acids encoding the hybrid proteins of this invention. These expression vectors are used for transforming the nucleic acids into host organisms and may also comprise sequences aiding in the expression of the nucleic acids in the host organism. The expression vectors may be plasmids, modified viruses, or DNA or RNA molecules, or other vectors useful in transformation systems known to the art.

By the methods of this invention, transformed cells are produced comprising the recombinant nucleic acid molecules capable of expressing the hybrid polypeptides of this invention. These may prokaryotic or eukaryotic cells from one-celled organisms, plants or animals. They may be bacterial cells from which the hybrid polypeptide may be harvested. Or, they may be plant cells which may be regenerated into plants from which the hybrid polypeptide may be harvested, or, such plant cells may be regenerated into fertile plants with seeds containing the nucleic acids encoding the hybrid polypeptide. In a preferred embodiment, such seeds contain modified starch comprising the payload polypeptide.

The term "modified starch" means the naturally-occurring starch has been modified to comprise the payload polypeptide.

A method of targeting digestion of a payload polypeptide to a particular phase of the digestive process, e.g., preventing degradation of a payload polypeptide in the stomach of an animal, is also provided comprising feeding the animal a modified starch of this invention comprising the payload polypeptide, whereby the polypeptide is protected by the starch from degradation in the stomach of the animal. Alternatively, the starch may be one known to be digested in the stomach to release the payload polypeptide there.

Preferred recombinant nucleic acid molecules of this invention comprise DNA encoding starch-encapsulating regions selected from the starch synthesizing gene sequences set forth in the tables hereof.

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Preferred plasmids of this invention are adapted for use with specific hosts.

Plasmids comprising a promoter, a plastid-targeting sequence, a nucleic acid sequence encoding a starch-encapsulating region, and a terminator sequence, are provided herein.

Such plasmids are suitable for insertion of DNA sequences encoding payload polypeptides and starch-encapsulating regions for expression in selected hosts.

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Plasmids of this invention can optionally include a spacer or a linker unit proximate the fusion site between nucleic acids encoding the SER and the nucleic acids encoding the payload polypeptide. This invention includes plasmids comprising promoters adapted for a prokaryotic or eukaryotic hosts. Such promoters may also be specifically adapted for expression in monocots or in dicots.

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A method of forming peptide-modified starch of this invention includes the steps of: supplying a plasmid having a promoter associated with a nucleic acid sequence encoding a starch-encapsulating region, the nucleic acid sequence encoding the starch-encapsulating region being connected to a nucleic acid region encoding a payload polypeptide, and transforming a host with the plasmid whereby the host expresses peptide-modified starch.

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This invention furthermore comprises starch-bearing grains comprising: an embryo, nutritive tissues; and, modified starch granules having encapsulated therein a protein that is

not endogenous to starch granules of said grain which are not modified. Such starchbearing grains may be grains wherein the embryo is a maize embryo, a rice embryo, or a wheat embryo.

All publications referred to herein are incorporated by reference to the extent not inconsistent herewith.

BRIEF DESCRIPTION OF THE DRAWINGS

- FIG. 1a shows the plasmid pEXS114 which contains the synthetic GFP (Green Fluorescent Protein) subcloned into pBSK from Stratagene.
 - FIG. 1b shows the plasmid pEXS115.
- 10 FIG. 2a. shows the waxy gene with restriction sites subcloned into a commercially available plasmid.
 - FIG. 2b shows the p ET-21A plasmid commercially available from Novagen having the GFP fragment from pEXS115 subcloned therein.
 - FIG. 3a shows pEXS114 subcloned into pEXSWX, and the GFP-FLWX map.
- FIG. 3b shows the GFP-Bam HIWX plasmid.
 - FIG. 4 shows the SGFP fragment of pEXS115 subcloned into pEXSWX, and the GFP-NcoWX map.
 - FIG. 5 shows a linear depiction of a plasmid that is adapted for use in monocots.
 - FIG. 6 shows the plasmid pEXS52.

FIG. 7 shows the six introductory plasmids used to form pEXS51 and pEX560."

FIG. 7a shows pEXS adh1. FIG. 7b shows pEXS adh1-nos3'. FIG. 7c shows pEXS33.

FIG. 7d shows pEXS10zp. FIG. 7e shows pEXS10zp-adh1. FIG. 7f shows pEXS10zp-adh1-nos3'.

FIGS. 8a and 8b show the plasmids pEXS50 and pEXS51, respectively, containing the MS-SIII gene which is a starch-soluble synthase gene.

FIG. 9a shows the plasmid pEXS60 which excludes the intron shown in pEXS50, and FIG. 9b shows the plasmid pEXS61 which excludes the intron shown in pEXS60.

DETAILED DESCRIPTION

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The present invention provides, broadly, a hybrid polypeptide, a method for making a hybrid polypeptide, and nucleic acids encoding the hybrid polypeptide. A hybrid polypeptide consists of two or more subparts fused together into a single peptide chain. The subparts can be amino acids or peptides or polypeptides. One of the subparts is a starch-encapsulating region. Hybrid polypeptides may thus be targeted into starch granules produced by organisms expressing the hybrid polypeptides.

A method of making the hybrid polypeptides within cells involves the preparation of a DNA construct comprising at least a fragment of DNA encoding a sequence which functions to bind the expression product of attached DNA into a granule of starch, ligated to a DNA sequence encoding the polypeptide of interest (the payload polypeptide). This construct is expressed within a eukaryotic or prokaryotic cell. The hybrid polypeptide can be used to produce purified protein or to immobilize a protein of interest within the protection of a starch granule, or to produce grain that contains foreign amino acids or peptides.

The hybrid polypeptide according to the present invention has three regions.

Payload Peptide	Central Site	Starch-encapsulating
(X)	(CS)*	region (SER)

X is any amino acid or peptide of interest.

* optional component.

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The gene for X can be placed in the 5' or 3' position within the DNA construct described below.

CS is a central site which may be a leaving site, a cleavage site, or a spacer, as is known to the art. A cleavage site is recognized by a cleaving enzyme. A cleaving enzyme is an enzyme that cleaves peptides at a particular site. Examples of chemicals and enzymes that have been employed to cleave polypeptides include thrombin, trypsin, cyanobromide, formic acid, hydroxyl amine, collagenase, and alasubtilisin. A spacer is a peptide that joins the peptides comprising the hybrid polypeptide. Usually it does not have any specific activity other than to join the peptides or to preserve some minimum distance or to influence the folding, charge or water acceptance of the protein. Spacers may be any peptide sequences not interfering with the biological activity of the hybrid polypeptide.

The starch-encapsulating region (SER) is the region of the subject polypeptide that has a binding affinity for starch. Usually the SER is selected from the group consisting of peptides comprising starch-binding regions of starch synthases and branching enzymes of plants, but can include starch binding domains from other sources such as glucoamylase and the like. In the preferred embodiments of the invention, the SER includes peptide products of genes that naturally occur in the starch synthesis pathway. This subset of preferred SERs is defined as starch-forming encapsulating regions (SFER). A further subset of SERs preferred herein is the specific starch-encapsulating regions (SSER) from the specific enzymes starch synthase (STS), granule-bound starch synthase (GBSTS) and branching enzymes (BE) of starch-bearing plants. The most preferred gene product from this set is the GBSTS. Additionally, starch synthase I and branching enzyme II are useful gene products. Preferably, the SER (and all the subsets discussed above) are truncated versions of the full length starch synthesizing enzyme gene such that the truncated portion includes the starch-encapsulating region.

The DNA construct for expressing the hybrid polypeptide within the host, broadly is as follows:

Promoter	Intron*	Transit Peptide	x	SER	Terminator
1		Coding Region*	<u> </u>		

* optional component. Other optional components can also be used.

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As is known to the art, a promoter is a region of DNA controlling transcription. Different types of promoters are selected for different hosts. Lac and T7 promoters work well in prokaryotes, the 35S CaMV promoter works well in dicots, and the polyubiquitin promoter works well in many monocots. Any number of different promoters are known to the art and can be used within the scope of this invention.

Also as is known to the art, an intron is a nucleotide sequence in a gene that does not code for the gene product. One example of an intron that often increases expression in monocots is the Adhl intron. This component of the construct is optional.

The transit peptide coding region is a nucleotide sequence that encodes for the translocation of the protein into organelles such as plastids. It is preferred to choose a transit peptide that is recognized and compatible with the host in which the transit peptide is employed. In this invention the plastid of choice is the amyloplast.

It is preferred that the hybrid polypeptide be located within the amyloplast in cells such as plant cells which synthesize and store starch in amyloplasts. If the host is a bacterial or other cell that does not contain an amyloplast, there need not be a transit peptide coding region.

A terminator is a DNA sequence that terminates the transcription.

X is the coding region for the payload polypeptide, which may be any polypeptide of interest, or chains of amino acids. It may have up to an entire sequence of a known polypeptide or comprise a useful fragment thereof. The payload polypeptide may be a

polypeptide, a fragment thereof, or biologically active protein which is an enzyme, hormone, growth factor, immunoglobulin, dye, etc. Examples of some of the payload polypeptides that can be employed in this invention include, but are not limited to, prolactin (PRL), serum albumin, growth factors and growth hormones, i.e., somatotropin. Serum albumins include bovine, ovine, equine, avian and human serum albumin. Growth factors include epidermal growth factor (EGF), insulin-like growth factor I (IGF-I), insulinlike growth factor II (IGF-II), fibroblast growth factor (FGF), transforming growth factor alpha (TGF-alpha), transforming growth factor beta (TGF-beta), nerve growth factor (NGF), platelet-derived growth factor (PDGF), and recombinant human insulin-like growth factors I (rHuIGF-I) and II (rHuIGF-II). Somatotropins which can be employed to practice this invention include, but are not limited to, bovine, porcine, ovine, equine, avian and human somatotropin. Porcine somatotropin includes delta-7 recombinant porcine somatotropin, as described and claimed in European Patent Application Publication No. 104,920 (Biogen). Preferred payload polypeptides are somatotropin, insulin A and B chains, calcitonin, beta endorphin, urogastrone, beta globin, myoglobin, human growth hormone, angiotensin, proline, proteases, beta-galactosidase, and cellulases.

The hybrid polypeptide, the SER region and the payload polypeptides may also include post-translational modifications known to the art such as glycosylation, acylation, and other modifications not interfering with the desired activity of the polypeptide.

Developing a Hybrid polypeptide

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The SER region is present in genes involved in starch synthesis. Methods for isolating such genes include screening from genomic DNA libraries and from cDNA libraries. Genes can be cut and changed by ligation, mutation agents, digestion, restriction and other such procedures, e.g., as outlined in Maniatis et al., Molecular Cloning, Cold Spring Harbor Labs, Cold Spring Harbor, N.Y. Examples of excellent starting materials for accessing the SER region include, but are not limited to, the following: starch synthases I, II, III, IV, Branching Enzymes I, IIA and B and granule-bound starch synthase (GBSTS). These genes are present in starch-bearing plants such as rice, maize, peas, potatoes, wheat, and the like. Use of a probe of SER made from genomic DNA or cDNA or mRNA or antibodies raised against the SER allows for the isolation and identification

of useful genes for cloning. The starch enzyme-encoding sequences may be modified as long as the modifications do not interfere with the ability of the SER region to encapsulate associated polypeptides.

When genes encoding proteins that are encapsulated into the starch granule are located, then several approaches to isolation of the SER can be employed, as is known to the art. One method is to cut the gene with restriction enzymes at various sites, deleting sections from the N-terminal end and allowing the resultant protein to express. The expressed truncated protein is then run on a starch gel to evaluate the association and dissociation constant of the remaining protein. Marker genes known to the art, e.g., green fluorescent protein gene, may be attached to the truncated protein and used to determine the presence of the marker gene in the starch granule.

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Once the SER gene sequence region is isolated it can be used in making the gene fragment sequence that will express the payload polypeptide encapsulated in starch. The SER gene sequence and the gene sequence encoding the payload polypeptide can be ligated together. The resulting fused DNA can then be placed in a number of vector constructs for expression in a number of hosts. The preferred hosts form starch granules in plastids, but the testing of the SER can be readily performed in bacterial hosts such as *E.coli*.

The nucleic acid sequence coding for the payload polypeptide may be derived from DNA, RNA, genomic DNA, cDNA, mRNA or may be synthesized in whole or in part. The sequence of the payload polypeptide can be manipulated to contain mutations such that the protein produced is a novel, mutant protein, so long as biological function is maintained.

When the payload polypeptide-encoding nucleic acid sequence is ligated onto the SER-encoding sequence, the gene sequence for the payload polypeptide is preferably attached at the end of the SER sequence coding for the N-terminus. Although the N-terminus end is preferred, it does not appear critical to the invention whether the payload polypeptide is ligated onto the N-terminus end or the C-terminus end of the SER. Clearly,

the method of forming the recombinant nucleic acid molecules of this invention, whether synthetically, or by cloning and ligation, is not critical to the present invention.

The central region of the hybrid polypeptide is optional. For some applications of the present invention it can be very useful to introduce DNA coding for a convenient protease cleavage site in this region into the recombinant nucleic acid molecule used to express the hybrid polypeptide. Alternatively, it can be useful to introduce DNA coding for an amino acid sequence that is pH-sensitive to form the central region. If the use of the present invention is to develop a pure protein that can be extracted and released from the starch granule by a protease or the like, then a protease cleavage site is useful. Additionally, if the protein is to be digested in an animal then a protease cleavage site may be useful to assist the enzymes in the digestive tract of the animal to release the protein from the starch. In other applications and in many digestive uses the cleavage site would be superfluous.

The central region site may comprise a spacer. A spacer refers to a peptide that joins the proteins comprising a hybrid polypeptide. Usually it does not have any specific activity other than to join the proteins, to preserve some minimum distance, to influence the folding, charge or hydrophobic or hydrophilic nature of the hybrid polypeptide.

Construct Development

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Once the ligated DNA which encodes the hybrid polypeptide is formed, then cloning vectors or plasmids are prepared which are capable of transferring the DNA to a host for expressing the hybrid polypeptides. The recombinant nucleic acid sequence of this invention is inserted into a convenient cloning vector or plasmid. For the present invention the preferred host is a starch granule-producing host. However, bacterial hosts can also be employed. Especially useful are bacterial hosts that have been transformed to contain some or all of the starch-synthesizing genes of a plant. The ordinarily skilled person in the art understands that the plasmid is tailored to the host. For example, in a bacterial host transcriptional regulatory promoters include lac, TAC, trp and the like. Additionally, DNA coding for a transit peptide most likely would not be used and a secretory leader that is upstream from the structural gene may be used to get the

polypeptide into the medium. Alternatively, the product is retained in the host and the host is lysed and the product isolated and purified by starch extraction methods or by binding the material to a starch matrix (or a starch-like matrix such as amylose or amylopectin, glycogen or the like) to extract the product.

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The preferred host is a plant and thus the preferred plasmid is adapted to be useful in a plant. The plasmid should contain a promoter, preferably a promoter adapted to target the expression of the protein in the starch-containing tissue of the plant. The promoter may be specific for various tissues such as seeds, roots, tubers and the like; or, it can be a constitutive promoter for gene expression throughout the tissues of the plant. Well-known promoters include the 10 kD zein (maize) promoter, the CAB promoter, patastin, 35S and 19S cauliflower mosaic virus promoters (very useful in dicots), the polyubiquitin promoter (useful in monocots) and enhancements and modifications thereof known to the art.

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The cloning vector may contain coding sequences for a transit peptide to direct the plasmid into the correct location. Examples of transit peptide-coding sequences are shown in the sequence tables. Coding sequences for other transit peptides can be used. Transit peptides naturally occurring in the host to be used are preferred. Preferred transit peptide coding regions for maize are shown in the tables and figures hereof. The purpose of the transit peptide is to target the vector to the correct intracellular area.

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Attached to the transit peptide-encoding sequence is the DNA sequence encoding the N-terminal end of the payload polypeptide. The direction of the sequence encoding the payload polypeptide is varied depending on whether sense or antisense transcription is desired. DNA constructs of this invention specifically described herein have the sequence encoding the payload polypeptide at the N- terminus end but the SER coding region can also be at the N-terminus end and the payload polypeptide sequence following. At the end of the DNA construct is the terminator sequence. Such sequences are well known in the art.

The cloning vector is transformed into a host. Introduction of the cloning vector, preferably a plasmid, into the host can be done by a number of transformation techniques known to the art. These techniques may vary by host but they include microparticle bombardment, micro injection, Agrobacterium transformation, "whiskers" technology (U.S. Patent Nos. 5,302,523 and 5,464,765), electroporation and the like. If the host is a plant, the cells can be regenerated to form plants. Methods of regenerating plants are known in the art. Once the host is transformed and the proteins expressed therein, the presence of the DNA encoding the payload polypeptide in the host is confirmable. The presence of expressed proteins may be confirmed by Western Blot or ELISA or as a result of a change in the plant or the cell.

Uses of Encapsulated Protein

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There are a number of applications of this invention. The hybrid polypeptide can be cleaved in a pure state from the starch (cleavage sites can be included) and pure protein can be recovered. Alternatively, the encapsulated payload polypeptide within the starch can be used in raw form to deliver protein to various parts of the digestive tract of the consuming animal ("animal" shall include mammals, birds and fish). For example if the starch in which the material is encapsulated is resistant to digestion then the protein will be released slowly into the intestine of the animal, therefore avoiding degradation of the valuable protein in the stomach. Amino acids such as methionine and lysine may be encapsulated to be incorporated directly into the grain that the animal is fed thus eliminating the need for supplementing the diet with these amino acids in other forms.

The present invention allows hormones, enzymes, proteins, proteinaceous nutrients and proteinaceous medicines to be targeted to specific digestive areas in the digestive tracts of animals. Proteins that normally are digested in the upper digestive tract encapsulated in starch are able to pass through the stomach in a nondigested manner and be absorbed intact or in part by the intestine. If capable of passing through the intestinal wall, the payload polypeptides can be used for medicating an animal, or providing hormones such as growth factors, e.g., somatotropin, for vaccination of an animal or for enhancing the nutrients available to an animal.

If the starch used is not resistant to digestion in the stomach (for example the sugary 2 starch is highly digestible), then the added protein can be targeted to be absorbed in the upper digestive tract of the animal. This would require that the host used to produce the modified starch be mutated or transformed to make sugary 2 type starch. The present invention encompasses the use of mutant organisms that form modified starch as hosts. Some examples of these mutant hosts include rice and maize and the like having sugary 1, sugary 2, brittle, shrunken, waxy, amylose extender, dull, opaque, and floury mutations, and the like. These mutant starches and starches from different plant sources have different levels of digestibility. Thus by selection of the host for expression of the DNA and of the animal to which the modified starch is fed, the hybrid polypeptide can be digested where it is targeted. Different proteins are absorbed most efficiently by different parts of the body. By encapsulating the protein in starch that has the selected digestibility, the protein can be supplied anywhere throughout the digestive tract and at specific times during the digestive process.

Another of the advantages of the present invention is the ability to inhibit or express differing levels of glycosylation of the desired polypeptide. The encapsulating procedure may allow the protein to be expressed within the granule in a different glycosylation state than if expressed by other DNA molecules. The glycosylation will depend on the amount of encapsulation, the host employed and the sequence of the polypeptide.

Improved crops having the above-described characteristics may be produced by genetic manipulation of plants known to possess other favorable characteristics. By manipulating the nucleotide sequence of a starch-synthesizing enzyme gene, it is possible to alter the amount of key amino acids, proteins or peptides produced in a plant. One or more genetically engineered gene constructs, which may be of plant, fungal, bacterial or animal origin, may be incorporated into the plant genome by sexual crossing or by transformation. Engineered genes may comprise additional copies of wildtype genes or may encode modified or allelic or alternative enzymes with new properties. Incorporation of such gene construct(s) may have varying effects depending on the amount and type of

gene(s) introduced (in a sense or antisense orientation). It may increase the plant's capacity to produce a specific protein, peptide or provide an improved amino acid balance.

Cloning Enzymes Involved in Starch Biosynthesis

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Known cloning techniques may be used to provide the DNA constructs of this invention. The source of the special forms of the SSTS, GBSTS, BE, glycogen synthase (GS), amylopectin, or other genes used herein may be any organism that can make starch or glycogen. Potential donor organisms are screened and identified. Thereafter there can be two approaches: (a) using enzyme purification and antibody/sequence generation following the protocols described herein; (b) using SSTS, GBSTS, BE, GS, amylopectin or other cDNAs as heterologous probes to identify the genomic DNAs for SSTS, GBSTS, BE, GS, amylopectin or other starch-encapsulating enzymes in libraries from the organism concerned. Gene transformation, plant regeneration and testing protocols are known to the art. In this instance it is necessary to make gene constructs for transformation which contain regulatory sequences that ensure expression during starch formation. These regulatory sequences are present in many small grains and in tubers and roots. For example these regulatory sequences are readily available in the maize endosperm in DNA encoding Granule Bound Starch Synthesis (GBSTS), Soluble Starch Synthases (SSTS) or Branching Enzymes (BE) or other maize endosperm starch synthesis pathway enzymes. These regulatory sequences from the endosperm ensure protein expression at the correct developmental time (e.g., ADPG pyrophosphorylase).

In this method we measure starch-binding constants of starch-binding proteins using native protein electrophoresis in the presence of suitable concentrations of carbohydrates such as glycogen or amylopectin. Starch-encapsulating regions can be elucidated using site-directed mutagenesis and other genetic engineering methods known to those skilled in the art. Novel genetically-engineered proteins carrying novel peptides or amino acid combinations can be evaluated using the methods described herein.

EXAMPLES

Example One:

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Method for Identification of Starch-encapsulating Proteins

Starch-Granule Protein Isolation:

Homogenize 12.5 g grain in 25 ml Extraction buffer (50 mM Tris acetate, pH 7.5, 1 mM EDTA, 1 mM DTT for 3 x 20 seconds in Waring blender with 1 min intervals between blending). Keep samples on ice. Filter through mira cloth and centrifuge at 6,000 rpm for 30 min. Discard supernatant and scrape off discolored solids which overlay white starch pellet. Resuspend pellet in 25 ml buffer and recentrifuge. Repeat washes twice more. Resuspend washed pellet in -20°C acetone, allow pellet to settle at -20°C. Repeat. Dry starch under stream of air. Store at -20°C.

Protein Extraction:

Mix 50 mg starch with 1 ml 2% SDS in eppendorf. Vortex, spin at 18,000 rpm, 5 min, 4°C. Pour off supernatant. Repeat twice. Add 1 ml sample buffer (4 ml distilled water, 1 ml 0.5 M Tris-HCl, pH 6.8, 0.8 ml glycerol, 1.6 ml 10% SDS, 0.4 ml B-mercaptoethanol, 0.2 ml 0.5% bromphenol blue). Boil eppendorf for 10 min with hole in lid. Cool, centrifuge 10,000 rpm for 10 min. Decant supernatant into new eppendorf. Boil for 4 minutes with standards. Cool.

SDS-Page Gels: (non-denaturing)

20		10% Resolve	4% Stack
	Acryl/Bis 40% stock	2.5 ml	1.0 ml
	1.5 M Tris pH 8.8	2.5 ml	-
	0.5 M Tris pH 8.8	-	2.5 ml
	10% SDS	100 μl	100 μl
25	Water	4.845 ml	6.34 ml
	Degas 15 min add fresh		
	10% Ammonium Persulfate	50 μl	50 μΙ
	TEMED	5μ1	10 μl

Mini-Protean II Dual Slab Cell; 3.5 ml of Resolve buffer per gel. 4% Stack is poured on top. The gel is run at 200V constant voltage. 10 x Running buffer (250 mM Tris, 1.92 M glycine, 1% SDS, pH 8.3).

Method of Measurement of Starch-Encapsulating Regions:

5 Solutions:

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Extraction Buffer: 50 mM Tris-acetate pH 7.5, 10 mM EDTA, 10%

sucrose, 2.5 mM DTT-fresh.

Stacking Buffer: 0.5 M Tris-HCl, pH 6.8

Resolve Buffer: 1.5 M Tris-HCl, pH 8.8

10 10 X Lower Electrode Buffer: 30.3 g Tris + 144 g Glycine qs to 1 L. (pH is ~8.3, no

adjustment). Dilute for use.

Upper Electrode Buffer: Same as Lower

Sucrose Solution: $18.66 \text{ g sucrose} + 100 \text{ ml } dH_2O$

30% Acryl/Bis Stock (2.67%C): 146 g acrylamide + 4 g bis + 350 ml dH₂O. Bring up

to 500 ml. Filter and store at 4 C in the dark for up

to 1 month.

15% Acryl/Bis Stock (20% C): 6 g acrylamide + 1.5 g bis + 25 ml dH_2O . Bring up

to 50 ml. Filter and store at 4 C in the dark for up to

1 month.

20 Riboflavin Solution: 1.4 g riboflavin + 100 ml dH₂O. Store in dark for up

to 1 month.

SS Assay mix: 25 mM Sodium Citrate, 25 mM Bicine-NaOH (pH

8.0), 2 mM EDTA, 1 mM DTT-fresh, 1 mM

Adenosine 5' Diphosphoglucose-fresh, 10 mg/ml rabbit

liver glycogen Type III-fresh.

Iodine Solution: 2 g iodine + 20 g KI, 0.1 N HCl up to 1 L.

Extract:

- 4 ml extraction buffer + 12 g endosperm. Homogenize.
- filter through mira cloth or 4 layers cheesecloth, spin 20,000 g (14,500 rpm, SM-24 rotor), 20 min., 4°C.
- 5 remove supernatant using a glass pipette.
 - · 0.85 ml extract + 0.1 ml glycerol + 0.05 ml 0.5% bromophenol blue.
 - vortex and spin 5 min. full speed microfuge. Use directly or freeze in liquid nitrogen and store at -80°C for up to 2 weeks.

Cast Gels:

Attach Gel Bond PAG film (FMC Industries, Rockland, ME) to (inside of) outer glass plate using two-sided scotch tape, hydrophilic side up. The tape and the film is lined up as closely and evenly as possible with the bottom of the plate. The film is slightly smaller than the plate. Squirt water between the film and the plate to adhere the film. Use a tissue to push out excess water. Set up plates as usual, then seal the bottom of the plates with tacky adhesive. The cassette will fit into the casting stand if the gray rubber is removed from the casting stand. The gel polymerizes with the film, and stays attached during all subsequent manipulations.

Cast 4.5% T resolve mini-gel (0.75 mm):

2.25 ml dH₂O

+ 3.75 ml sucrose solution

+ 2.5 ml resolve buffer

+ 1.5 ml 30% Acryl/Bis stock

+ various amounts of glycogen for each gel (i.e., 0 - 1.0%)

DEGAS 15 MIN.

25 + 50 μl 10% APS

+ 5 µl TEMED

POLYMERIZE FOR 30 MIN. OR OVERNIGHT

Cast 3.125 % T stack:

1.59 ml dH₂O

- + 3.75 ml sucrose solution
- + 2.5 ml stack buffer
- + 2.083 ml 15% Acryl/Bis stock

DO NOT DEGAS

5 15 μl 10% APS

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- + 35 µl riboflavin solution
- + 30 µl TEMED

POLYMERIZE FOR 2.5 HOURS CLOSE TO A LIGHT BULB

cool in 4°C before pulling out combs. Can also not use combs, and just cast a centimeter of stacker.

The foregoing procedure:

- · Can run at different temperatures; preincubate gels and solutions.
- Pre-run for 15 min. at 200 V
- Load gel: 7 μl per well, or 115 μl if no comb.
- Run at 140 V until dye front is close to bottom. Various running temperatures are achieved by placing the whole gel rig into a water bath. Can occasionally stop the run to insert a temperature probe into the gel.
 - Enzyme assay: Cut gels off at dye front. Incubate in SS. Assay mix overnight at room temperature with gentle shaking. Rinse gels with water. Flood with I2/KI solution.
 - Take pictures of the gels on a light box, and measure the pictures. Rm = mm from top of gel to the active band/mm from top of gel to the bottom of the gel where it was cut (where the dye front was). Plot % glycogen vs. 1/Rm. The point where the line intersects the x axis is -K (where y=0).

25 Testing and evaluation protocol for SER region length:

Following the procedure above for selection of the SER region requires four basic steps. First DNA encoding a protein having a starch-encapsulation region must be selected. This can be selected from known starch-synthesizing genes or starch-binding genes such as genes for amylases, for example. The protein must be extracted. A number of protein extraction techniques are well known in the art. The protein may be treated

with proteases to form protein fragments of different lengths. The preferred fragments have deletions primarily from the N-terminus region of the protein. The SER region is located nearer to the C-terminus end than the N-terminus end. The protein is run on the gels described above and affinity for the gel matrix is evaluated. Higher affinity shows more preference of that region of the protein for the matrix. This method enables comparison of different proteins to identify the starch-encapsulating regions in natural or synthetic proteins.

Example Two:

SER Fusion Vector:

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The following fusion vectors are adapted for use in *E.coli*. The fusion gene that was attached to the probable SER in these vectors encoded for the green fluorescent protein (GFP). Any number of different genes encoding for proteins and polypeptides could be ligated into the vectors. A fusion vector was constructed having the SER of waxy maize fused to a second gene or gene fragment, in this case GFP.

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pEXS114 (see FIG. 1a): Synthetic GFP (SGFP) was PCR-amplified from the plasmid HBT-SGFP (from Jen Sheen; Dept. of Molecular Biology; Wellman 11, MGH; Boston, MA 02114) using the primers EXS73 (5'-GACTAGTCATATG GTG AGC AAG GGC GAG GAG-3') [SEQ ID NO:1] and EXS74 (5'-CTAGATCTTCATATG CTT GTA CAG CTC GTC CAT GCC-3') [SEQ ID NO:2]. The ends of the PCR product were polished off with T DNA polymerase to generate blunt ends; then the PCR product was digested with *Spe* I. This SGFP fragment was subcloned into the *Eco*RV-*Spe* I sites of pBSK (Stratagene at 11011 North Torrey Pines Rd. La Jolla, Ca.) to generate pEXS114.

25

pEXS115 [see FIG. 1b]: Synthetic GFP (SGFP) was PCR-amplified from the plasmid HBT-SGFP (from Jen Sheen) using the primers EXS73 (see above) and EXS75 (5'-CTAGATCTTGGCCATGGC CTT GTA CAG CTC GTC CAT GCC-3') [SEQ ID NO:3]. The ends of the PCR product were polished off with T DNA polymerase to generate blunt ends; then the PCR product was digested with Spe I. This SGFP fragment was subcloned into the EcoRV-Spe I sites of pBSK (Stratagene) generating pEXS115.

pEXSWX (see FIG. 2a): Maize WX subcloned *NdeI-Not* I into pET-21a (see FIG. 2b). The genomic DNA sequence and associated amino acids from which the mRNA sequence can be generated is shown in TABLES 1a and 1b below and alternatively the DNA listed in the following tables could be employed.

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TABLE 1a DNA Sequence and Deduced Amino Acid Sequence of the waxy Gene in Maize [SEQ ID NO:4 and SEQ ID NO:5]

			-
10	LOCUS DEFINITION	ZMWAXY Zea mays	4800 bp DNA PLN waxy (wx+) locus for UDP-glucose starch glycosyl
	ACCESSION KEYWORDS	transfer X03935 M glycosyl	24258 transit peptide:
15	SOURCE	maize.	ose starch glycosyl transferase; waxy locus.
	ORGANISM	Zea mays	
	OROHNIDH	Fukaryot	a; Plantae; Embryobionta; Magnoliophyta; Liliopsida;
		Commelia	idae; Cyperales; Poaceae.
	REFERENCE		s 1 to 4800)
20	AUTHORS	Kloesgen	,R.B., Gierl,A., Schwarz-Sommer,Z. and Saedler,H.
	TITLE	Molecula	r analysis of the waxy locus of Zea mays
	JOURNAL	Mol. Gen	. Genet. 203, 237-244 (1986)
	STANDARD	full aut	omatic
25	COMMENT	NCBI gi:	
25	FEATURES		Location/Qualifiers
	source		14800
		• ·	/organism="Zea mays"
	repeat_	_region	283287
30	renest	region	/note="direct repeat 1" 288292
•	rebear_	_region	/note="direct repeat 1"
	repeat	region	293297
			/note="direct repeat 1"
	repeat	region	298302
35	•		/note="direct repeat 1"
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	_		/note="GC stretch (pot. regulatory factor binding
	site)"		•
40	misc_fe	eature	442468
70	site)"		/note="GC stretch (pot. regulatory factor binding
	misc fe		760 701
	zc_re	acure	768782
	site)"		/note="GC stretch (pot. regulatory factor binding
45	misc fe	ature	810822
	_		/note="GC stretch (pot. regulatory factor binding
	site)"		, (Last asymmetry and a series of
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50	_		<pre>/note="target duplication site (Ac7)"</pre>
50	CAAT_si	-	821828
	TATA_si	-	867873
	misc_fe	ature	887900
	site)"		/note="GC stretch (pot. regulatory factor binding
55	misc fe	ature	901
-			/note="transcriptional start site"
	exon		9011080
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			•

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       KIYGPVAGTDYRDNQLRFSLLCQAALEAPRILSLNNNPYFSGPYGEDVVFVCNDWHTG
20
       PLSCYLKSNYOSHGIYRDAKTAFCIHNISYOGRFAFSDYPELNLPERFKSSFDFIDGY
       EKPVEGRKINWMKAGILEADRVLTVSPYYAEELISGIARGCELDNIMRLTGITGIVNG
       MDVSEWDPSRDKYIAVKYDVSTAVEAKALNKEALQAEVGLPVDRNIPLVAFIGRLEEQ
25
       KGPDVMAAAIPQLMEMVEDVQIVLLGTGKKKFERMLMSAEEKFPGKVRAVVKFNAALA
       HHIMAGADVLAVTSRFEPCGLIQLQGMRYGTPCACASTGGLVDTIIEGKTGFHMGRLS
30
       VDCNVVEPADVKKVATTLQRAIKVVGTPAYEEMVRNCMIQDLSWKGPAKNWENVLLSL
                             GVAGGEPGVEGEEIAPLAKENVAAP"
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            polyA_site
polyA_site
                            4618
                            4625
       BASE COUNT
                       935 A
                               1413 C
                                        1447 G
                                                 1005 T
       ORIGIN
               1 CAGCGACCTA TTACACAGCC CGCTCGGGCC CGCGACGTCG GGACACATCT TCTTCCCCCT
25
              61 TTTGGTGAAG CTCTGCTCGC AGCTGTCCGG CTCCTTGGAC GTTCGTGTGG CAGATTCATC
             121 TGTTGTCTCG TCTCCTGTGC TTCCTGGGTA GCTTGTGTAG TGGAGCTGAC ATGGTCTGAG
30
             181 CAGGCTTAAA ATTTGCTCGT AGACGAGGAG TACCAGCACA GCACGTTGCG GATTTCTCTG
             301 CGATGCGGTG GTGAGCAGAG CAGCAACAGC TGGGCGGCCC AACGTTGGCT TCCGTGTCTT
35
             361 CGTCGTACGT ACGCGCGCGC CGGGGACACG CAGCAGAGAG CGGAGAGCGA GCCGTGCACG
             421 GGGAGGTGGT GTGGAAGTGG AGCCGCGCGC CCGGCCGCCC GCGCCCGGTG GGCAACCCAA
40
             481 AAGTACCCAC GACAAGCGAA GGCGCCAAAG CGATCCAAGC TCCGGAACGC AACAGCATGC
             541 GTCGCGTCGG AGAGCCAGCC ACAAGCAGCC GAGAACCGAA CCGGTGGGCG ACGCGTCATG
             601 GGACGGACGC GGGCGACGCT TCCAAACGGG CCACGTACGC CGGCGTGTGC GTGCGTGCAG
45
             661 ACGACAAGCC AAGGCGAGGC AGCCCCCGAT CGGGAAAGCG TTTTGGGCGC GAGCGCTGGC
            721 GTGCGGGTCA GTCGCTGGTG CGCAGTGCCG GGGGGAACGG GTATCGTGGG GGGCGCGGGC
50
             781 GGAGGAGAGC GTGGCGAGGG CCGAGAGCAG CGCGCGGCCG GGTCACGCAA CGCGCCCCAC
            841 GTACTGCCCT CCCCCTCCGC GCGCGCTAGA AATACCGAGG CCTGGACCGG GGGGGGGCCC
            901 CGTCACATCC ATCCATCGAC CGATCGATCG CCACAGCCAA CACCACCCGC CGAGGCGACG
55
            961 CGACAGCCGC CAGGAGGAAG GAATAAACTC ACTGCCAGCC AGTGAAGGGG GAGAAGTGTA
            1021 CTGCTCCGTC GACCAGTGCG CGCACCGCCC GGCAGGGCTG CTCATCTCGT CGACGACCAG
60
           1081 GTTCTGTTCC GTTCCGATCC GATCCGATCC TGTCCTTGAG TTTCGTCCAG ATCCTGGCGC
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3361 GGAGATGGTG GAGGACGTGC AGATCGTTCT GCTGGTACGT GTGCGCCGGC CGCCACCCGG 3421 CTACTACATG CGTGTATCGT TCGTTCTACT GGAACATGCG TGTGAGCAAC GCGATGGATA 5 3481 ATGCTGCAGG GCACGGGCAA GAAGAAGTTC GAGCGCATGC TCATGAGCGC CGAGGAGAAG 3541 TTCCCAGGCA AGGTGCGCGC CGTGGTCAAG TTCAACGCGG CGCTGGCGCA CCACATCATG 3601 GCCGGCGCG ACGTGCTCGC CGTCACCAGC CGCTTCGAGC CCTGCGGCCT CATCCAGCTG 10 3661 CAGGGGATGC GATACGGAAC GGTACGAGAG AAAAAAAAA TCCTGAATCC TGACGAGAGG 3721 GACAGAGACA GATTATGAAT GCTTCATCGA TTTGAATTGA TTGATCGATG TCTCCCGCTG 15 3781 CGACTCTTGC AGCCCTGCGC CTGCGCGTCC ACCGGTGGAC TCGTCGACAC CATCATCGAA 3841 GGCAAGACCG GGTTCCACAT GGGCCGCCTC AGCGTCGACG TAAGCCTAGC TCTGCCATGT 3901 TCTTTCTTCT TTCTTTCTGT ATGTATGTAT GAATCAGCAC CGCCGTTCTT GTTTCGTCGT 20 3961 CGTCCTCTC TCCCAGTGTA ACGTCGTGGA GCCGGCGGAC GTCAAGAAGG TGGCCACCAC 4021 ATTGCAGCGC GCCATCAAGG TGGTCGGCAC GCCGGCGTAC GAGGAGATGG TGAGGAACTG 25 4081 CATGATCCAG GATCTCTCCT GGAAGGTACG TACGCCCGCC CCGCCCGCC CCGCCAGAGC 4141 AGAGCGCCAA GATCGACCGA TCGACCGACC ACACGTACGC GCCTCGCTCC TGTCGCTGAC 4201 CGTGGTTTAA TTTGCGAAAT GCGCAGGGCC CTGCCAAGAA CTGGGAGAAC GTGCTGCTCA 30 4261 GCCTCGGGGT CGCCGGCGGC GAGCCAGGGG TCGAAGGCGA GGAGATCGCG CCGCTCGCCA 4321 AGGAGAACGT GGCCGCGCCC TGAAGAGTTC GGCCTGCAGG GCCCCTGATC TCGCGCGTGG 35 4381 TGCAAAGATG TTGGGACATC TTCTTATATA TGCTGTTTCG TTTATGTGAT ATGGACAAGT 4501 TAATAAGCGC ATGAACTAAT TGCTTGCGTG TGTAGTTAAG TACCGATCGG TAATTTTATA 40 4561 TTGCGAGTAA ATAAATGGAC CTGTAGTGGT GGAGTAAATA ATCCCTGCTG TTCGGTGTTC 4621 TTATCGCTCC TCGTATAGAT ATTATAGA GTACATTTTT CTCTCTCTGA ATCCTACGTT 45 4681 TGTGAAATTT CTATATCATT ACTGTAAAAT TTCTGCGTTC CAAAAGAGAC CATAGCCTAT 4741 CTTTGGCCCT GTTTGTTTCG GCTTCTGGCA GCTTCTGGCC ACCAAAAGCT GCTGCGGACT 11

TABLE 1b

DNA Sequence and Deduced Amino Acid Sequence in waxy Gene in Rice [SEO ID NO:6 and SEO ID NO:7]

```
5
         Locus
                      OSWX
                                   2542 bp
                                               RNA
                                                               PLN
         DEFINITION
                     O.sativa Waxy mRNA.
         ACCESSION
                     X62134 S39554
         KEYWORDS
                     glucosyltransferase; starch biosynthesis; waxy gene.
         SOURCE
                     rice.
  10
           ORGANISM
                     Oryza sativa
                     Eukaryota; Plantae; Embryobionta; Magnoliophyta; Liliopsida;
                     Commelinidae; Cyperales; Poaceae.
         REFERENCE
                         (bases 1 to 2542)
           AUTHORS
                     Okayaki, R.J.
  15
           TITLE
                     Direct Submission
           JOURNAL
                     Submitted (12-SEP-1991) to the EMBL/GenBank/DDBJ databases.
         R.J.
                     Okayaki, University of Florida, Dep of Vegetable Crops, 1255
                     Fifield Hall, 514 IFAS, Gainesville, Florida 32611-0514, USA
 20
           STANDARD
                     full automatic
         REFERENCE
                     2 (bases 1 to 2542)
          AUTHORS
                     Okagaki, R.J.
                     Nucleotide sequence of a long cDNA from the rice waxy gene
           TITLE
          JOURNAL
                     Plant Mol. Biol. 19, 513-516 (1992)
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                              /codon start=1
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       DVLGGLPPAMAANGHRVMVISPRYDQYKDAWDTSVVAEIKVADRYERVRFFHCYKRGV
       DRVFIDHPSFLEKVWGKTGEKIYGPDTGVDYKDNQMRFSLLCQAALEAPRILNLNNNP
       YFKGTYGEDVVFVCNDWHTGPLASYLKNNYQPNGIYRNAKVAFCIHNISYQGRFAFED
50
       YPELNLSERFRSSFDFIDGYDTPVEGRKINWMKAGILEADRVLTVSPYYAEELISGIA
       RGCELDNIMRLTGITGIVNGMDVSEWDPSKDKYITAKYDATTAIEAKALNKEALQAEA
55
       GLPVDRKIPLIAFIGRLEEQKGPDVMAAAIPELMQEDVQIVLLGTGKKKFEKLLKSME
       EKYPGKVRAVVKFNAPLAHLIMAGADVLAVPSRFEPCGLIQLQGMRYGTPCACASTGG
       LVDTVIEGKTGFHMGRLSVDCKVVEPSDVKKVAATLKRAIKVVGTPAYEEMVRNCMNQ
60
                             DLSWKGPAKNWENVLLGLGVAGSAPGIEGDEIAPLAKENVAAP"
            3'UTR
                             2283..2535
            polyA_site
                             2535
       BASE COUNT
                       610 A
                                 665 C
                                          693 G
       ORIGIN
65
               1 GAATTCAGTG TGAAGGAATA GATTCTCTTC AAAACAATTT AATCATTCAT CTGATCTGCT
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61 CAAAGCTCTG TGCATCTCCG GGTGCAACGG CCAGGATATT TATTGTGCAG TAAAAAAATG 121 TCATATCCCC TAGCCACCCA AGAAACTGCT CCTTAAGTCC TTATAAGCAC ATATGGCATT 5 181 GTAATATATA TGTTTGAGTT TTAGCGACAA TTTTTTTAAA AACTTTTGGT CCTTTTTATG 241 AACGTTTTAA GTTTCACTGT CTTTTTTTT CGAATTTTAA ATGTAGCTTC AAATTCTAAT 10 361 GAAAACCAGT TCAAATTCTT TTTAGGCTCA CCAAACCTTA AACAATTCAA TTCAGTGCAG 421 AGATCTTCCA CAGCAACAGC TAGACAACCA CCATGTCGGC TCTCACCACG TCCCAGCTCG 15 481 CCACCTCGGC CACCGGCTTC GGCATCGCCG ACAGGTCGGC GCCGTCGTCG CTGCTCCGCC 601 TGACGACCAG CGCGCGCG ACGCCCAAGC AGCAGCGGTC GGTGCAGCGT GGCAGCCGGA 20 661 GGTTCCCCTC CGTCGTCGTG TACGCCACCG GCGCCGGCAT GAACGTCGTG TTCGTCGGCG 721 CCGAGATGGC CCCCTGGAGC AAGACCGGCG GCCTCGGTGA CGTCCTCGGT GGCCTCCCCC 25 781 CTGCCATGGC TGCGAATGGC CACAGGGTCA TGGTGATCTC TCCTCGGTAC GACCAGTACA 841 AGGACGCTTG GGATACCAGC GTTGTGGCTG AGATCAAGGT TGCAGACAGG TACGAGAGGG 901 TGAGGTTTTT CCATTGCTAC AAGCGTGGAG TCGACCGTGT GTTCATCGAC CATCCGTCAT 30 961 TCCTGGAGAA GGTTTGGGGA AAGACCGGTG AGAAGATCTA CGGACCTGAC ACTGGAGTTG 1021 ATTACAAAGA CAACCAGATG CGTTTCAGCC TTCTTTGCCA GGCAGCACTC GAGGCTCCTA 35 1081 GGATCCTAAA CCTCAACAAC AACCCATACT TCAAAGGAAC TTATGGTGAG GATGTTGTGT 1141 TCGTCTGCAA CGACTGGCAC ACTGGCCCAC TGGCGAGCTA CCTGAAGAAC AACTACCAGC 1201 CCAATGGCAT CTACAGGAAT GCAAAGGTTG CTTTCTGCAT CCACAACATC TCCTACCAGG 40 1261 GCCGTTTCGC TTTCGAGGAT TACCCTGAGC TGAACCTCTC CGAGAGGTTC AGGTCATCCT 1321 TCGATTTCAT CGACGGGTAT GACACGCCGG TGGAGGGCAG GAAGATCAAC TGGATGAAGG 45 1381 CCGGAATCCT GGAAGCCGAC AGGGTGCTCA CCGTGAGCCC GTACTACGCC GAGGAGCTCA 1441 TCTCCGGCAT CGCCAGGGGA TGCGAGCTCG ACAACATCAT GCGGCTCACC GGCATCACCG 1501 GCATCGTCAA CGGCATGGAC GTCAGCGAGT GGGATCCTAG CAAGGACAAG TACATCACCG 50 1561 CCAAGTACGA CGCAACCACG GCAATCGAGG CGAAGGCGCT GAACAAGGAG GCGTTGCAGG 1621 CGGAGGCGGG TCTTCCGGTC GACAGGAAAA TCCCACTGAT CGCGTTCATC GGCAGGCTGG 55 1681 AGGAACAGAA GGGCCCTGAC GTCATGGCCG CCGCCATCCC GGAGCTCATG CAGGAGGACG 1741 TCCAGATCGT TCTTCTGGGT ACTGGAAAGA AGAAGTTCGA GAAGCTGCTC AAGAGCATGG 1801 AGGAGAAGTA TCCGGGCAAG GTGAGGGCGG TGGTGAAGTT CAACGCGCCG CTTGCTCATC 60 1861 TCATCATGGC CGGAGCCGAC GTGCTCGCCG TCCCCAGCCG CTTCGAGCCC TGTGGACTCA 1921 TCCAGCTGCA GGGGATGAGA TACGGAACGC CCTGTGCTTG CGCGTCCACC GGTGGGCTCG 65 1981 TGGACACGGT CATCGAAGGC AAGACTGGTT TCCACATGGG CCGTCTCAGC GTCGACTGCA 2041 AGGTGGTGGA GCCAAGCGAC GTGAAGAAGG TGGCGGCCAC CCTGAAGCGC GCCATCAAGG

		2101	TCGTCGGCAC	GCCGGCGTAC	GAGGAGATGG	TCAGGAACTG	CATGAACCAG	GACCTCTCCT
		2161	GGAAGGGGCC	TGCGAAGAAC	TGGGAGAATG	TGCTCCTGGG	CCTGGGCGTC	GCCGGCAGCG
5		2221	CGCCGGGGAT	CGAAGGCGAC	GAGATCGCGC	CGCTCGCCAA	GGAGAACGTG	GCTGCTCCTT
		2281	GAAGAGCCTG	AGATCTACAT	ATGGAGTGAT	TAATTAATAT	AGCAGTATAT	GGATGAGAGA
		2341	CGAATGAACC	AGTGGTTTGT	TTGTTGTAGT	GAATTTGTAG	CTATAGCCAA	TTATATAGGÇ
10		2401	TAATAAGTTT	GATGTTGTAC	TCTTCTGGGT	GTGCTTAAGT	ATCTTATCGG	ACCCTGAATT
		2461	TATGTGTGTG	GCTTATTGCC	AATAATATTA	AGTAATAAAG	GGTTTATTAT	ATTATTATAT
15		2521	ATGTTATATT	АТАСТААААА	AA			
	11							

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TABLE 2

DNA Sequence and Deduced Amino Acid Sequence of the Soluble Starch Synthase IIa Gene in Maize [SEQ ID NO:8 and SEQ ID NO:9]

FILE NAME : MSS2C.SEQ SEQUENCE : NORMAL 2007 BP

CODON TABLE : UNIV.TCN

SEQUENCE REGION: 1 - 2007

TRANSLATION REGION: 1 - 2007

*** DNA TRANSLATION ***

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30	49 17	GAC D	GCC A	GCC A	AGG '	TTG (CCC P	CGC (GCT (CGG R	CGC 1	AAT O N	GCG C	TC 1 V	rcc A s	AAA C	GG R	96 32
	97 33	AGG R	GAT D	CCT	CTT L	CAG (CCG P	GTC V	GGC G	CGG R	TAC (GGC 1	rcc (GCG A	ACG (GA P	AAC N	144 48
	145		G GCG	C AGG	ACC T	GGC G	GCC A	GCG A	TCC S	TGC C	CAG Q	AAC N	GCC A	GCA A	TTG L	GCG A	GAC D	192 64
35	193 65		GAC E	ATC I	GTT V	GAG E	ATC I	AAG K	TCC S	ATC I	GTC V	GCC A	GCG A	CCG P	CCG P	ACG T	AGC S	240 80
	241 81		A GTO	G AAC K	TTC F	CCA P	GGG G	CGC R	GGG G	CTA L	CAG Q	GAT D	GAT D	CCT P	TCC S	CTC L	TGG W	288 96
40	289 91		AT:	A GCA	A CCG	GAG E	ACT T	GTC V	CTC L	CCA P	GCC A	CCG P	AAG K	CCA P	CTG L	CAT H	GAA E	336 112
	33°		G CC	r gco	G GTT V	GAC D	GGA G	GAT D	TCA S	AAT N	GGA G	ATT	GCA A	CCT P	CCT P	ACA T	GTT V	384 128
	38: 12:				A GTA	CAG	GAC E	G GCC	ACT T	TGG	GAT D	TTC F	AAG K	AAA K	TAC Y	ATC I	GGT G	432 144
45	43.	3 T T	T GA	C GA	G CCI	GAC	GAA	A GCG	; AAG	GAT	GAT	TCC	AGG	GTT	GGT	GCA	GAT	480

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             GAT GCT GGT TCT TTT GAA CAT TAT GGG ACA ATG ATT CTG GGC CTT TGT
        481
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                                                                          C
                                                                                176
             GGG GAG AAT GTT ATG AAC GTG ATC GTG GTG GCT GCT GAA TGT TCT CCA
        529
  5
                                                                                576
        177
                                  N
                                      V
                                          Ι
                                              V
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                                                              E
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             TGG TGC AAA ACA GGT GGT CTT GGA GAT GTT GTG GGA GCT TTA CCC AAG
        577
        193
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             GCT TTA GCG AGA AGA GGA CAT CGT GTT ATG GTT GTG GTA CCA AGG TAT
        625
                                                                                672
        209
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             GGG GAC TAT GTG GAA GCC TTT GAT ATG GGA ATC CGG AAA TAC TAC AAA
        673
                                                                                720
        225
                  D
                      Y
                          V
                              Ε
                                  Α
                                      F
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                                                                                768
        241
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                                              N
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                                                                                256
             GGA GTC GAC TTT GTG TTC ATT GAT GCC TCT TTC CGG CAC CGT CAA GAT
 15
                                                                                816
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                             V
                                  F
                                      Ι
                                          D
                                             Α
                                                 S
                                                     F
                                                         R
                                                             Н
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                                                                         D
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        817
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                                          Q
                                             Ε
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                                                     М
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             TTT TGC AAG GTT GCT GTT GAG GTT CCT TGG CAC GTT CCA TGC GGT GGT
        865
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        289
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            GTG TGC TAC GGA GAT GGA AAT TTG GTG TTC ATT GCC ATG AAT TGG CAC
        913
                                                                                960
        305
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             TTA ATG CAG TAC ACT CGC TCC GTC CTC GTC ATA CAT AAC ATC GGC CAC
       1009
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             CAG GGC CGT GGT CCT GTA CAT GAA TTC CCG TAC ATG GAC TTG CTG AAC
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             ACT AAC CTT CAA CAT TTC GAG CTG TAC GAT CCC GTC GGT GGC GAG CAC
       1105
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       1201
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       1249
35
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       1489
            GGA CAG AAG GGC GTG GAC ATC ATC GGG GAC GCG ATG CCG TGG ATC GCG
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             G
                 Q
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	1537 513	GG C	CAG Q	GAC D	GTG V	CAG	CTG L	GTG V	ATG M	CTC L	GGC G	ACC T	GGC G	CCA P	CCT	GAC D	CTG L	1584 528
	1585 529				_	¥	••		£	R	E	н	₽	N	K	V	CGC R	1632 544
5	1633	GGG	TGG	GTC	GGG	TTC	TCG	GTC	CTA	ATG	GTG	CAT	CGC	ATC	ACG	CCG	GGC	1680
	545	G	W	V	G	F	S	V	L	M	V	H	R	I	T	P	G	560
	1681	GCC	AGC	GTG	CTG	GTG	ATG	CCC	TCC	CGC	TTC	GCC	GGC	GGG	CTG	AAC	CAG	1728
	561	A	S	V	L	V	M	P	S	R	F	A	G	G	L	N	Q	576
10	1729	CTC	TAC	GCG	ATG	GCA	TAC	GGC	ACC	GTC	CCT	GTG	GTG	CAC	GCC	GTG	GGC	1776
	577	L	Y	A	M	A	Y	G	T	V	P	V	V	H	A	V	G	592
	1777	GGG	CTC	AGG	GAC	ACC	GTG	GCG	CCG	TTC	GAC	CCG	TTC	GGC	GAC	GCC	GGG	1824
	593	G	L	R	D	T	V	A	P	F	D	P	F	G	D	A	G	608
	1825	CTC	GGG	TGG	ACT	TTT	GAC	CGC	GCC	GAG	GCC	AAC	AAG	CTG	ATC	GAG	GTG	1872
	609	L	G	W	T	F	D	R	A	E	A	N	K	L	I	E	V	624
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	625	L	S	H	C	L	D	T	Y	R	N	Y	E	E	S	W	K	640
	1921	AGT	CTC	CAG	GCG	CGC	GGC	ATG	TCG	CAG	AAC	CTC	AGC	TGG	GAC	CAC	GCG	1968
	641	S	L	Q	A	R	G	M	S	Q	N	L	S	W	D	H	A	656
20	1969 657	GCT A	GAG E	CTC L	TAC Y	GAG E	GAC D	GTC V	CTT L	GTC V	AAG K	TAC Y	CAG Q	TGG W				2007 669

TABLE 3 DNA Sequence and Deduced Amino Acid Sequence of The Soluble Starch Synthase IIb Gene in Maize [SEQ ID NO:10 and SEQ ID NO: 11]

25 FILE NAME : MSS3FULL.DNA SEQUENCE : NORMAL 2097 BP

CODON TABLE : UNIV.TCN

SEQUENCE REGION: 1 - 2097

TRANSLATION REGION : 1 - 2097

*** DNA TRANSLATION ***

30	1	ATG M	CCG P	GGG G	GCA A	ATC I	TCT S	TCC S	TCG S	TCG S	TCG S	GCT A	TTT F	CTC	CTC L	CCC P	GTC V	48 16
	49	GCG	TCC	TCC	TCG	CCG	CGG	CGC	AGG	CGG	GGC	AGT	GTG	GGT	GCT	GCT	CTG	96
	17	A	S	S	S	P	R	R	R	R	G	S	V	G	A	A	L	32
35	97	CGC	TCG	TAC	GGC	TAC	AGC	GGC	GCG	GAG	CTG	CGG	TTG	CAT	TGG	GCG	CGG	144
	33	R	S	Y	G	Y	S	G	A	E	L	R	L	H	W	A	R	48
	145	CGG	GGC	CCG	CCT	CAG	GAT	GGA	GCG	GCG	TCG	GTA	CGC	GCC	GCA	GCG	GCA	192
	49	R	G	P	P	Q	D	G	A	A	S	V	R	A	A	A	A	64
	193	CCG	GCC	GGG	GGC	GAA	AGC	GAG	GAG	GCA	GCG	AAG	AGC	TCC	TCC	TCG	TCC	240
	65	P	A	G	G	E	S	E	E	A	A	K	S	S	S	S	S	80
40	241	CAG	GCG	GGC	GCT	GTT	CAG	GGC	AGC	ACG	GCC	AAG	GCT	стс	GAT	ጥርጥ	СĆТ	200

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            TCA CCT CCC AAT CCT TTG ACA TCT GCT CCG AAG CAA AGT CAG AGC GCT
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            GCA ATG CAA AAC GGA ACG AGT GGG GGC AGC AGC ACC GCC GCG
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        385
            CCG GTG TCC GGA CCC AAA GCT GAT CAT CCA TCA GCT CCT GTC ACC AAG
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        433
            AGA GAA ATC GAT GCC AGT GCG GTG AAG CCA GAG CCC GCA GGT GAT GAT
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            GCT AGA CCG GTG GAA AGC ATA GGC ATC GCT GAA CCG GTG GAT GCT AAG
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                                                                              176
            GCT GAT GCA GCT CCG GCT ACA GAT GCG GCG GCG AGT GCT CCT TAT GAC
                                                                              576
       177
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            AGG GAG GAT AAT GAA CCT GGC CCT TTG GCT GGG CCT AAT GTG ATG AAC
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       193
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                                    G
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                                                                              208
            GTC GTC GTG GCT TCT GAA TGT GCT CCT TTC TGC AAG ACA GGT GGC
       625
                                                                              672
                                    Ε
                                        С
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            CTT GGA GAT GTC GTG GGT GCT TTG CCT AAG GCT CTG GCG AGG AGA GGA
       673
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       721
            CAC CGT GTT ATG GTC GTG ATA CCA AGA TAT GGA GAG TAT GCC GAA GCC
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            CGG GAT TTA GGT GTA AGG AGA CGT TAC AAG GTA GCT GGA CAG GAT TCA
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            GAA GTT ACT TAT TTT CAC TCT TAC ATT GAT GGA GTT GAT TTT GTA TTC
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            GTA GAA GCC CCT CCC TTC CGG CAC CGG CAC AAT AAT ATT TAT GGG GGA
       865
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       913
            GAA AGA TTG GAT ATT TTG AAG CGC ATG ATT TTG TTC TGC AAG GCC GCT
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           GTT GAG GTT CCA TGG TAT GCT CCA TGT GGC GGT ACT GTC TAT GGT GAT
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            GGC AAC TTA GTT TTC ATT GCT AAT GAT TGG CAT ACC GCA CTT CTG CCT
       1009
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            GTC TAT CTA AAG GCC TAT TAC CGG GAC AAT GGT TTG ATG CAG TAT GCT
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            CGC TCT GTG CTT GTG ATA CAC AAC ATT GCT CAT CAG GGT CGT GGC CCT
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       1153
            GTA GAC GAC TTC GTC AAT TTT GAC TTG CCT GAA CAC TAC ATC GAC CAC
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      1201
            TTC AAA CTG TAT GAC AAC ATT GGT GGG GAT CAC AGC AAC GTT TTT GCT
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      1249
            GCG GGG CTG AAG ACG GCA GAC CGG GTG GTG ACC GTT AGC AAT GGC TAC
       417
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      1297
            ATG TGG GAG CTG AAG ACT TCG GAA GGC GGG TGG GGC CTC CAC GAC ATC
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45
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	449		N AAC	Q Q	AAC N	GAC D	TGC W	AAG K	CTC L	CAC Q	GG(ATC I	GTG V	AAC N	GGC G	ATC I	GAC D	139 46
	1393 465	ATG M	AGC S	GAG E	TGG W	AAC N	P CCC	GCT A	GTG V	GAC D	GTC V	CAC H	CTC L	CAC H	TCC	GAC D	GAC D	144 48
5	1441 481	TAC Y	ACC T	AAC N	TAC Y	ACG T	TTC	GAG E	ACG T	CTG L	GAC D	ACC T	GGC G	AAG K	CGG R	CAG Q	TGC C	1488 490
·	1489	AAG	GCC	GCC	CTG	CAG	CGG	CAG	CTG	GGC	CTG	CAG	GTC	CGC	GAC	GAC	GTG	1536
	497	K	A	A	L	Q	R	Q	L	G	L	Q	V	R	D	D	V	512
10	1537 513	CCA P	CTG L	ATC I	GGG G	TTC	ATC I	GGG G	CGG R	CTG L	GAC D	CAC H	CAG Q	AAG K	GGC G	GTG V	GAC D	1584 528
	1585	ATC	ATC	GCC	GAC	GCG	ATC	CAC	TGG	ATC	GCG	GGG	CAG	GAC	GTG	CAG	CTC	632
	529	I	I	A	D	A	I	H	W	I	A	G	Q	D	V	Q	L	544
	1633	GTG	ATG	CTG	GGC	ACC	GGG	CGG	GCC	GAC	CTG	GAG	GAC	ATG	CTG	CGG	CGG	1680
	545	V	M	L	G	T	G	R	A	D	L	E	D	M	L	R	R	560
15	1681	TTC	GAG	TCG	GAG	CAC	AGC	GAC	AAG	GTG	CGC	GCG	TGG	GTG	GGG	TTC	TCG	1728
	561	F	E	S	E	H	S	D	K	V	R	A	W	V	G	F	S	576
	1729	gtg	CCC	CTG	GCG	CAC	CGC	ATC	ACG	GCG	GGC	GCG	GAC	ATC	CTG	CTG	ATG	1776
	577	V	P	L	A	H	R	I	T	A	G	A	D	I	L	L	M	592
20	1777	CCG	TCG	CGG	TTC	GAG	CCG	TGC	GGG	CTG	AAC	CAG	CTC	TAC	GCC	ATG	GCG	1824
	593	P	S	R	F	E	P	C	G	L	N	Q	L	Y	A	M	A	608
	1825	TAC	GGG	ACC	GTG	CCC	GTG	GTG	CAC	GCC	GTG	GGG	GGG	CTC	CGG	GAC	ACG	1872
	609	Y	G	T	V	P	V	V	H	A	V	G	G	L	R	D	T	624
	1873	GTG	GCG	CCG	TTC	GAC	CCG	TTC	AAC	GAC	ACC	GGG	CTC	GGG	TGG	ACG	TTC	1920
	625	V	A	P	F	D	P	F	N	D	T	G	L	G	W	T	F	640
25	1921	GAC	CGC	GCG	GAG	GCG	AAC	CGG	ATG	ATC	GAC	GCG	CTC	TCG	CAC	TGC	CTC	1968
	641	D	R	A	E	A	N	R	M	I	D	A	L	S	H	C	L	656
	1969 657	ACC T	ACG T	TAC Y	CGG R	AAC N	TAC Y	AAG K	GAG E	AGC S	TGG W	CGC R	GCC A	TGC C	AGG R	GCG A		2016
30	2017 673	GGC G	ATG M	GCC A	GAG E	GAC D	CTC L	AGC S	TGG W	GAC D	CAC H	GCC A	GCC A	GTG V	CTG L			2064 688
	2065 689	GAC D	GTG V	CTC L	GTC .	AAG K	GCG A	AAG K	TAC Y	CAG Q	TGG W	TGA *			-	-	-	2097 699

TABLE 4

DNA and Deduced Amino Acid Sequence of The Soluble Starch Synthase I Gene in Maize [SEQ ID NO:12; SEQ ID NO: 13]

FILE NAME : MSS1FULL.DNA SEQUENCE : NORMAL 1752 BP

CODON TABLE : UNIV.TCN

35

SEQUENCE REGION: 1 - 1752

40 TRANSLATION REGION: 1 - 1752

	TGC Cys 700	, va.	C GC	G GAG a Glu	G CTO	G AGO Ser 705	ME	G GAG	G GGG	G CC	C GC O Al	a Pr	G CG	C CC	G CTO	G CCA Pro 715		48
5	Pro	C GCC	G CTO	G CTO	G GC0 1 Ala 720	PEC	CCG Pro	CTO Le	C GTO	G CC0 1 Pro 729	o GT	C TT y Ph	C CTO	C GCC	G CCC a Pro 730	G CCG Pro		96
	GCC Ala	GAC Glu	CCC Pro	735	. Gry	GAG Glu	CCG Pro	GCA Ala	A TCC A Ser 740	Thi	G CCC	G CCC	G CC	C GTC Val 745	Pro	GAC Asp	. 1	.44
10	GCC Ala	GGC Gly	7 Leu 750	r GTA	GAC Asp	CTC Leu	GGT Gly	CTC Leu 755	GLU	A CCI	GAZ Glu	A GGC	G ATT 7 Ile 760	Ala	GAA	GGT	1	.92
15	TCC Ser	Ile 765	. vəř	AAC Asn	ACA Thr	GTA Val	GTT Val 770	vai	GCA Ala	AGI Ser	GAC Glu	G CAF 1 Glr 775	ı Asp	TCT Ser	GAG	ATT	. 2	40
	GTG Val 780	AGI	GGA Gly	AAG Lys	GAG Glu	CAA Gln 785	GCT Ala	CGA Arg	GCT Ala	AAA Lys	GTA Val 790	. Thr	CAA Gln	AGC Ser	ATT Ile	GTC Val 795	28	88
20	TTT Phe	GTA Val	ACC	GGC	GAA Glu 800	GCT Ala	TCT Ser	CCT Pro	TAT Tyr	GCA Ala 805	Lys	TCT	GGG Gly	GGT Gly	CTA Leu 810	GGA Gly	3:	36
	GAT Asp	GTT Val	TGT Cys	GGT Gly 815	TCA Ser	TTG Leu	CCA Pro	GTT Val	GCT Ala 820	Leu	GCT Ala	GCT Ala	CGT Arg	GGT Gly 825	CAC His	CGT Arg	38	84
25	GTG Val	ATG Met	GTT Val 830	Val	ATG Met	CCC Pro	AGA Arg	TAT Tyr 835	TTA Leu	AAT Asn	GGT Gly	ACC Thr	TCC Ser 840	GAT Asp	AAG Lys	AAT Asn	43	32
30	TAT Tyr	GCA Ala 845	AAT Asn	GCA Ala	TTT Phe	TAC Tyr	ACA Thr 850	GAA Glu	AAA Lys	CAC His	ATT Ile	CGG Arg 855	ATT Ile	CCA Pro	TGC Cys	TTT Phe	48	30
	GGC Gly 860	GGT Gly	GAA Glu	CAT His	GAA Glu	GTT Val 865	ACC Thr	TTC Phe	TTC Phe	CAT His	GAG Glu 870	TAT Tyr	AGA Arg	GAT Asp	TCA Ser	GTT Val 875	52	28
35	GAC Asp	TGG Trp	GTG Val	TTT Phe	GTT Val 880	GAT Asp	CAT His	CCC Pro	TCA Ser	TAT Tyr 885	CAC His	AGA Arg	CCT Pro	GGA Gly	AAT Asn 890	TTA Leu	57	'6
	TAT Tyr	GGA Gly	GAT Asp	AAG Lys 895	TTT Phe	GGT Gly	GCT Ala	TTT Phe	GGT Gly 900	GAT Asp	AAT Asn	CAG Gln	TTC Phe	AGA Arg 905	TAC Tyr	ACA Thr	62	4
40	CTC Leu	CTT Leu	TGC Cys 910	TAT Tyr	GCT Ala	GCA Ala	Cys	GAG Glu 915	GCT Ala	CCT Pro	TTG Leu	ATC Ile	CTT Leu 920	GAA Glu	TTG Leu	GGA Gly	67	2
45	GGA Gly	TAT Tyr 925	ATT Ile	TAT Tyr	GGA Gly	GIN !	AAT Asn 930	TGC Cys	ATG Met	TTT Phe	GTT Val	GTC Val 935	AAT Asn	GAT Asp	TGG Trp	CAT His	72	0
	GCC Ala 940	AGT Ser	CTA Leu	GTG Val	PFO	GTC (Val 1 945	CTT (Leu)	CTT Leu	GCT Ala	Ala	AAA Lys 950	TAT Tyr	AGA Arg	CCA Pro	Tyr	GGT Gly 955	76	8
50	GTT (rat . ryr :	AAA Lys	ASD	TCC (Ser) 960	CGC P Arg S	AGC : Ser :	ATT Ile	Leu	GTA Val 965	ATA Ile	CAT His	AAT Asn	Leu	GCA Ala 970	CAT His	816	6

	CAG GGT GTA GAG CCT GCA AGC ACA TAT CCT GAC CTT GGG TTG CCA CCT Gln Gly Val Glu Pro Ala Ser Thr Tyr Pro Asp Leu Gly Leu Pro Pro 975 980 985	864
5	GAA TGG TAT GGA GCT CTG GAG TGG GTA TTC CCT GAA TGG GCG AGG AGG Glu Trp Tyr Gly Ala Leu Glu Trp Val Phe Pro Glu Trp Ala Arg Arg 990 995 1000	912
	CAT GCC CTT GAC AAG GGT GAG GCA GTT AAT TTT TTG AAA GGT GCA GTT His Ala Leu Asp Lys Gly Glu Ala Val Asn Phe Leu Lys Gly Ala Val 1005 1010 1015	960
10	GTG ACA GCA GAT CGA ATC GTG ACT GTC AGT AAG GGT TAT TCG TGG GAG Val Thr Ala Asp Arg Ile Val Thr Val Ser Lys Gly Tyr Ser Trp Glu 1025 1030 1035	1008
15	GTC ACA ACT GCT GAA GGT GGA CAG GGC CTC AAT GAG CTC TTA AGC TCC Val Thr Thr Ala Glu Gly Gly Gln Gly Leu Asn Glu Leu Leu Ser Ser 1040 1045 1050	1056
٠	AGA AAG AGT GTA TTA AAC GGA ATT GTA AAT GGA ATT GAC ATT AAT GAT Arg Lys Ser Val Leu Asn Gly Ile Val Asn Gly Ile Asn Ile Asn Asp 1055 1060 1065	1104
20	TGG AAC CCT GCC ACA GAC AAA TGT ATC CCC TGT CAT TAT TCT GTT GAT Trp Asn Pro Ala Thr Asp Lys Cys Ile Pro Cys His Tyr Ser Val Asp 1070 1080	1152
	GAC CTC TCT GGA AAG GCC AAA TGT AAA GGT GCA TTG CAG AAG GAG CTG Asp Leu Ser Gly Lys Ala Lys Cys Lys Gly Ala Leu Gln Lys Glu Leu 1085 1090 1095	1200
25	GGT TTA CCT ATA AGG CCT GAT GTT CCT CTG ATT GGC TTT ATT GGA AGG Gly Leu Pro Ile Arg Pro Asp Val Pro Leu Ile Gly Phe Ile Gly Arg 1100 1115	1248
30	TTG GAT TAT CAG AAA GGC ATT GAT CTC ATT CAA CTT ATC ATA CCA GAT Leu Asp Tyr Gln Lys Gly Ile Asp Leu Ile Gln Leu Ile Ile Pro Asp 1120 1125 1130	1296
	1135 Phe Val Met Leu Gly Ser Gly Asp Pro 1145	1344
35	GAG CTT GAA GAT TGG ATG AGA TCT ACA GAG TCG ATC TTC AAG GAT AAA Glu Leu Glu Asp Trp Met Arg Ser Thr Glu Ser Ile Phe Lys Asp Lys 1150 1155 1160	1392
	TTT CGT GGA TGG GTT GGA TTT AGT GTT CCA GTT TCC CAC CGA ATA ACT Phe Arg Gly Trp Val Gly Phe Ser Val Pro Val Ser His Arg Ile Thr 1165 1170 1175	1440
40	1180 Led Led Met Pro Ser Arg Phe Glu Pro Cys Gly 1180 1185 1190 1195	1488.
45	1200 1205 1210	1536
	GCA ACT GGG GGC CTT AGA GAT ACC GTG GAG AAC TTC AAC CCT TTC GGT Ala Thr Gly Gly Leu Arg Asp Thr Val Glu Asn Phe Asn Pro Phe Gly 1215 1220 1225	1584
50	GAG AAT GGA GAG CAG GGT ACA GGG TGG GCA TTC GCA CCC CTA ACC ACA Glu Asn Gly Glu Gln Gly Thr Gly Trp Ala Phe Ala Pro Leu Thr Thr 1230 1240	1632

		1	245				12	250	LG A	sii C	ys A	sn I	le T 255	yr I	le G	AG GGA ln Gly	1680
5	Ac Tl	CA C nr G 260	AA G1 Ln Va	rc ca	CC CT		A AC y Az 65	GG GC	CT AA	AT G	LU A	CG AG la Ai 270	GG Ci rg H.	AT G1 is Va	rc Al	AA AGA ys Arg 127	1728
	C: Le	TT CA	AC G1 Ls Va	G GG	уРг	A TG O Cy 80	C CG	C TC	A								1752
10	(2	!) IN	FORM	ATIO	N FO	R SE	מו ס	NO:	13.								
				SEQ (UENC A) L B) T	E CH ENGT: YPE: OPOL	ARAC H: 5 ami	TERI 84 a no a	STIC mino cid	S: aci	.ds	•					
15			(ii)	MOL	ECUL	E TY	PE:	prot	ein								
			(xi)	SEQ	UENC	E DE	SCRI	PTIO	N: S	EQ I	D NO	:13:					
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				_					,				4.	5		o Asp	
	Ala	Gly 50	/ Leu	ı Gly	/ Asp	Leu	Gly 55	Leu ;	Glu	Pro	Gl:	Gly 60	y Ile	e Ala	a Glu	ı Gly	
25						, 0					/:	•				Ile 80	
	Val	. Val	. Gly	. Tàs	Glu 85	Gln	Ala	. Arg	Ala	Lys 90	Va]	Thr	Glr	ser	Ile 95	Val	
30			*						105					110		Gly	
	Asp	Val	Cys 115	Gly	Ser	Leu	Pro	Val 120	Ala	Leu	Ala	Ala	Arg 125		His	Arg	
	Val	Met 130	Val	Val	Met	Pro	Arg 135	Tyr	Leu	Asn	Gly	Thr 140	Ser	Asp	Lys	Asn	
35	Tyr 145	Ala	Asn	Ala	Phe	Tyr 150	Thr	Glu	Lys	His	Ile 155	Arg	Ile	Pro	Cys	Phe 160	
	Gly	Gly	Glu	His	Glu 165	Val	Thr	Phe	Phe	His 170	Glu	Tyr	Arg	Asp	Ser 175	Val	•
40	Asp	Trp	Val	Phe 180	Val	Asp	His	Pro	Ser 185	Tyr	His	Arg	Pro	Gly 190		Leu	
	Tyr	Gly	Asp 195	Lys	Phe	Gly	Ala	Phe 200	Gly	Asp	Asn	Gln	Phe 205		Tyr	Thr	
	Leu	Leu 210	Cys	Tyr	Ala	Ala	Cys 215	Glu	Ala	Pro	Leu	Ile 220		Glu	Leu	Gly	
45	Gly 225	Tyr	Ile	Tyr	Gly	Gln 230	Asn	Cys	Met	Phe	Val 235	Val	Asn	Asp	Trp	His 240	

	Ala	Ser	Leu	Val	Pro 245	Val	Leu	Leu	Ala	Ala 250	Lys	Tyr	Arg	Pro	Tyr 255	Gly
	Val	Tyr	Lys	Asp 260	Ser	Arg	Ser	Ile	Leu 265	Val	Ile	His	Asn	Leu 270	Ala	His
5	Gln	Gly	Val 275	Glu	Pro	Ala	Ser	Thr 280	Tyr	Pro	Asp	Leu	Gly 285	Leu	Pro	Pro
	Glu	Trp 290	Tyr	Gly	Ala	Leu	Glu 295	Trp	Val	Phe	Pro	Glu 300	Trp	Ala	Arg	Arg
10	His 305	Ala	Leu	Asp	Lys	Gly 310	Glu	Ala	Val	Asn	Phe 315	Leu	ГЛа	Gly	Ala	Val 320
	Val	Thr	Ala	Asp	Arg 325	Ile	Val	Thr	Val	Ser 330	Lys	Gly	Tyr	Ser	Trp 335	Glu
	Val	Thr	Thr	Ala 340	Glu	Gly	Gly	Gln	Gly 345	Leu	Asn	Glu	Leu	Leu 350	Ser	Ser
15	Arg	Lys	Ser 355	Val	Leu	Asn	Gly	Ile 360	Val	Asn	Gly	Ile	Asp 365	Ile	Asn	Asp
	Trp	Asn 370	Pro	Ala	Thr	Asp	Lys 375	Cys	Ile	Pro	Cys	His 380	Tyr	Ser	Val	Asp
20	Asp 385	Leu	Ser	Gly	Lys	Ala 390	Lys	Cys	Lys	Gly	Ala 395	Leu	Gln	Lys	Glu	Leu 400
	Gly	Leu	Pro	Ile	Arg 405	Pro	Asp	Val	Pro	Leu 410	Ile	Gly	Phe	Ile	Gly 415	Arg
	Leu	Asp	Tyr	Gln 420	Lys	Gly	Ile	Asp	Leu 425	Ile	Gln	Leu	Ile	Ile 430	Pro	Asp
25	Leu	Met	Arg 435	Glu	Asp	Val	Gln	Phe 440	Val	Met	Leu	Gly	Ser 445	Gly	Asp	Pro
	Glu	Leu 450	Glu	Asp	Trp	Met	Arg 455	Ser	Thr	Glu	Ser	Ile 460	Phe	Lys	Asp	Lys
30	Phe 465	Arg	Gly	Trp	Val	Gly 470	Phe	Ser	Val	Pro	Val 475	Ser	His	Arg	Ile	Thr 480
	Ala	Gly	Cys	Asp	Ile 485	Leu	Leu	Met	Pro	Ser 490	Arg	Phe	Glu	Pro	Cys 495	Gly
	Leu	Asn	Gln	Leu 500	Tyr	Ala	Met	Gln	Tyr 505	Gly	Thr	Val	Pro	Val 510	Val	His
35	Ala	Thr	Gly 515	Gly	Leu	Arg	Asp	Thr 520	Val	Glu	Asn	Phe	Asn 525	Pro	Phe	Gly
	Glu	Asn 530	Gly	Glu	Gln	Gly	Thr 535	Gly	Trp	Ala	Phe	Ala 540	Pro	Leu	Thr	Thr
40	Glu 545	Asn	Met	Phe	Val	Asp 550	Ile	Ala	Asn	Cys	Asn 555	Ile	Tyr	Ile	Gln	Gly 560
	Thr	Gln	Val	Leu	Leu 565	Gly	Arg	Ala	Asn	Glu 570	Ala	Arg	His	Val	Lys 575	Arg
	Leu	His	Val	Gly 580	Pro	Cys	Arg	*								

TABLE 5 mRNA Sequence and Deduced Amino Acid Sequence of The Maize Branching Enzyme II Gene and the Transit Peptide [SEO ID NO:14 and SEO ID NO:15]

_		·
5	ACCESSION L	ZEGLUCTRN 2725 bp ss-mRNA PLN Drn starch branching enzyme II mRNA, complete cds. 08065 ,4-alpha-glucan branching enzyme; amylo-transglycosylase;
10	SOURCE ZE ORGANISM ZE	ea mays cDNA to mRNA.
15	REFERENCE 1 AUTHORS FI TITLE St	chkaryota; Plantae; Embryobionta; Magnoliophyta; Liliopsida; commelinidae; Cyperales; Poaceae. (bases 1 to 2725) isher, D.K., Boyer, C.D. and Hannah, L.C. carch branching enzyme II from maize endosperm
20	STANDARD fu	ant Physiol. 102, 1045-1046 (1993) Ill automatic BI gi: 168482 Location/Qualifiers 12725 /cultivar="W64Ax182E"
25	sig_pepti	<pre>/dev_stage="29 days post pollenation" /tissue_type="endosperm" /organism="2ea mays"</pre>
30	CDS	912490 /EC_number="2.4.1.18" /note="NCBI gi: 168483" /codon_start=1 /product="starch branching enzyme II"
35	/translation="	MAFRVSGAVLGGAVRAPRLTGGGEGSLVFRHTGLFLTRGARVGC
33		KAVMVPEGENDGLASRADSAQFQSDELEVPDISEETTCGAGVAD
		DGQKIFQIDPMLQGYKYHLEYRYSLYRRIRSDIDEHEGGLEAFS
40		ITYREWAPGAFSAALVGDVNNWDPNADRMSKNEFGVWEIFLPNN
		/RMDTPSGIKDSIPAWIKYSVQAPGEIPYDGIYYDPPEEVKYVF
45		THVGMSSPEPKINTYVNFRDEVLPRIKKLGYNAVQIMAIQEHS
		PSSRFGTPEDLKSLIDRAHELGLLVLMDVVHSHASSNTLDGLNG
	FDGTDTHYFHSGPRG	HHWMWDSRLFNYGNWEVLRFLLSNARWWLEEYKFDGFRFDGVT
50	SMMYTHHGLQVTFTG	NFNEYFGFATDVDAVVYLMLVNDLIHGLYPEAVTIGEDVSGMP
	TFALPVHDGGVGFDY	RMHMAVADKWIDLLKQSDETWKMGDIVHTLTNRRWLEKCVTYA
55	ESHDQALVGDKTIAF	WLMDKDMYDFMALDRPSTPTIDRGIALHKMIRLITMGLGGEGY
		t FPRGPQRLPSGKFIPGNNNSYDKCRRRFDLGDADYLRYHGMQE
60		TSDHQYISRKHEEDKVIVFEKGDLVFVFNFHCNNSYFDYRIGC
60		LFGGFSRIHHAAEHFTADCSHDNRPYSFSVYTPSRTCVVYAPV E"
	mat_peptide	/codon start=1
65	BASE COUNT	/product="starch branching enzyme II" 727 A 534 C 715 G 749 T

ORIGIN 1 GGCCCAGAGC AGACCCGGAT TTCGCTCTTG CGGTCGCTGG GGTTTTAGCA TTGGCTGATC 61 AGTTCGATCC GATCCGGCTG CGAAGGCGAG ATGGCGTTCC GGGTTTCTGG GGCGGTGCTC 121 GGTGGGGCCG TAAGGGCTCC CCGACTCACC GGCGGCGGGG AGGGTAGTCT AGTCTTCCGG 5 181 CACACCGGCC TCTTCTTAAC TCGGGGTGCT CGAGTTGGAT GTTCGGGGAC GCACGGGGCC 241 ATGCGCGCGG CGGCCGCGC CAGGAAGGCG GTCATGGTTC CTGAGGGCGA GAATGATGGC 301 CTCGCATCAA GGGCTGACTC GGCTCAATTC CAGTCGGATG AACTGGAGGT ACCAGACATT 361 TCTGAAGAGA CAACGTGCGG TGCTGGTGTG GCTGATGCTC AAGCCTTGAA CAGAGTTCGA 421 GTGGTCCCCC CACCAAGCGA TGGACAAAAA ATATTCCAGA TTGACCCCAT GTTGCAAGGC 10 481 TATAAGTACC ATCTTGAGTA TCGGTACAGC CTCTATAGAA GAATCCGTTC AGACATTGAT 541 GAACATGAAG GAGGCTTGGA AGCCTTCTCC CGTAGTTATG AGAAGTTTGG ATTTAATGCC 601 AGCGCGGAAG GTATCACATA TCGAGAATGG GCTCCTGGAG CATTTTCTGC AGCATTGGTG 661 GGTGACGTCA ACAACTGGGA TCCAAATGCA GATCGTATGA GCAAAAATGA GTTTGGTGTT 721 TGGGAAATTT TTCTGCCTAA CAATGCAGAT GGTACATCAC CTATTCCTCA TGGATCTCGT 15 781 GTAAAGGTGA GAATGGATAC TCCATCAGGG ATAAAGGATT CAATTCCAGC CTGGATCAAG 841 TACTCAGTGC AGGCCCCAGG AGAAATACCA TATGATGGGA TTTATTATGA TCCTCCTGAA 901 GAGGTAAAGT ATGTGTTCAG GCATGCGCAA CCTAAACGAC CAAAATCATT GCGGATATAT 961 GAAACACATG TCGGAATGAG TAGCCCGGAA CCGAAGATAA ACACATATGT AAACTTTAGG 1021 GATGAAGTCC TCCCAAGAAT AAAAAAACTT GGATACAATG CAGTGCAAAT AATGGCAATC 20 1081 CAAGAGCACT CATATTATGG AAGCTTTGGA TACCATGTAA CTAATTTTTT TGCGCCAAGT 1141 AGTCGTTTTG GTACCCCAGA AGATTTGAAG TCTTTGATTG ATAGAGCACA TGAGCTTGGT 1201 TTGCTAGTTC TCATGGATGT GGTTCATAGT CATGCGTCAA GTAATACTCT GGATGGGTTG 1261 AATGGTTTG ATGGTACAGA TACACATTAC TTTCACAGTG GTCCACGTGG CCATCACTGG 1321 ATGTGGGATT CTCGCCTATT TAACTATGGG AACTGGGAAG TTTTAAGATT TCTTCTCTCC 25 1381 AATGCTAGAT GGTGGCTCGA GGAATATAAG TTTGATGGTT TCCGTTTTGA TGGTGTGACC 1441 TCCATGATGT ACACTCACCA CGGATTACAA GTAACATTTA CGGGGAACTT CAATGAGTAT 1501 TTTGGCTTTG CCACCGATGT AGATGCAGTG GTTTACTTGA TGCTGGTAAA TGATCTAATT 1561 CATGGACTTT ATCCTGAGGC TGTAACCATT GGTGAAGATG TTAGTGGAAT GCCTACATTT 1621 GCCCTTCCTG TTCACGATGG TGGGGTAGGT TTTGACTATC GGATGCATAT GGCTGTGGCT 30 1681 GACAAATGGA TTGACCTTCT CAAGCAAAGT GATGAAACTT GGAAGATGGG TGATATTGTG 1741 CACACACTGA CAAATAGGAG GTGGTTAGAG AAGTGTGTAA CTTATGCTGA AAGTCATGAT 1801 CAAGCATTAG TCGGCGACAA GACTATTGCG TTTTGGTTGA TGGACAAGGA TATGTATGAT 1861 TTCATGGCCC TCGATAGACC TTCAACTCCT ACCATTGATC GTGGGATAGC ATTACATAAG 1921 ATGATTAGAC TTATCACAAT GGGTTTAGGA GGAGAGGGCT ATCTTAATTT CATGGGAAAT 35 1981 GAGTTTGGAC ATCCTGAATG GATAGATTTT CCAAGAGGTC CGCAAAGACT TCCAAGTGGT 2041 AAGTTTATTC CAGGGAATAA CAACAGTTAT GACAAATGTC GTCGAAGATT TGACCTGGGT 2101 GATGCAGACT ATCTTAGGTA TCATGGTATG CAAGAGTTTG ATCAGGCAAT GCAACATCTT 2161 GAGCAAAAAT ATGAATTCAT GACATCTGAT CACCAGTATA TTTCCCGGAA ACATGAGGAG 2221 GATAAGGTGA TTGTGTTCGA AAAGGGAGAT TTGGTATTTG TGTTCAACTT CCACTGCAAC 40 2281 AACAGCTATT TTGACTACCG TATTGGTTGT CGAAAGCCTG GGGTGTATAA GGTGGTCTTG 2341 GACTCCGACG CTGGACTATT TGGTGGATTT AGCAGGATCC ATCACGCAGC CGAGCACTTC 2401 ACCGCCGACT GTTCGCATGA TAATAGGCCA TATTCATTCT CGGTTTATAC ACCAAGCAGA 2461 ACATGTGTCG TCTATGCTCC AGTGGAGTGA TAGCGGGGTA CTCGTTGCTG CGCGGCATGT 2521 GTGGGGCTGT CGATGTGAGG AAAAACCTTC TTCCAAAACC GGCAGATGCA TGCATGCATG 45 2581 CTACAATAAG GTTCTGATAC TTTAATCGAT GCTGGAAAGC CCATGCATCT CGCTGCGTTG 2641 TCCTCTCTAT ATATATAGA CCTTCAAGGT GTCAATTAAA CATAGAGTTT TCGTTTTTCG 2701 СТТТССТААА ААААААААА ААААА 11

TABLE 6 mRNA Sequence and Deduced Amino Acid Sequence of the Maize Branching Enzyme I and the Transit Peptide [SEQ ID NO:16 and SEQ ID NO:17]

```
LOCUS
                   MZEBEI
                                 2763 bp ss-mRNA
                                                             PLN
       DEFINITION
                   Maize mRNA for branching enzyme-I (BE-I).
55
       ACCESSION
                   D11081
       KEYWORDS
                   branching enzyme-I.
       SOURCE
                   Zea mays L. (inbred Oh43), cDNA to mRNA.
         ORGANISM Zea mays
                   Eukaryota; Plantae; Embryobionta; Magnoliophyta; Liliopsida;
60
                   Commelinidae; Liliopsida.
      REFERENCE
                     (bases 1 to 2763)
        AUTHORS
                   Baba, T., Kimura, K., Mizuno, K., Etoh, H., Ishida, Y., Shida, O. and
                   Arai, Y.
```

50

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Sequence conservation of the catalytic regions of Amylolytic.
          TITLE
                    enzymes in maize branching enzyme-I
          JOURNAL
                    Biochem. Biophys. Res. Commun. 181, 87-94 (1991)
          STANDARD
                    full automatic
  5
        COMMENT
                    Submitted (30-APR-1992) to DDBJ by: Tadashi Baba
                    Institute of Applied Biochemistry
                    University of Tsukuba
                    Tsukuba, Ibaraki 305
                    Japan
 10
                    Phone: 0298-53-6632
                            0298-53-6632.
                    Fax:
                    NCBI gi: 217959
        FEATURES
                             Location/Qualifiers
             source
                             1..2763
 15
                             /organism="Zea mays"
             CDS
                             <1..2470
                             /note="NCBI gi: 217960"
                             /codon start=2
                             /product="branching enzyme-I precursor"
 20
        translation="LCLVSPSSSPTPLPPPRRSRSHADRAAPPGIAGGGNVRLSVLSV/
        QCKARRSGVRKVKSKFATAATVQEDKTMATAKGDVDHLPIYDLDPKLEIFKDHFRYRM
25
       KRFLEQKGSIEENEGSLESFSKGYLKFGINTNEDGTVYREWAPAAQEAELIGDFNDWN
        GANHKMEKDKFGVWSIKIDHVKGKPAIPHNSKVKFRFLHGGVWVDRIPALIRYATVDA
       SKFGAPYDGVHWDPPASERYTFKHPRPSKPAAPRIYEAHVGMSGEKPAVSTYREFADN
30
       VLPRIRANNYNTVQLMAVMEHSYYASFGYHVTNFFAVSSRSGTPEDLKYLVDKAHSLG
       LRVLMDVVHSHASNNVTDGLNGYDVGQSTQESYFHAGDRGYHKLWDSRLFNYANWEVL
35
       RFLLSNLRYWLDEFMFDGFRFDGVTSMLYHHHGINVGFTGNYQEYFSLDTAVDAVVYM
       MLANHLMHKLLPEATVVAEDVSGMPVLCRPVDEGGVGFDYRLAMAIPDRWIDYLKNKD
       DSEWSMGEIAHTLTNRRYTEKCIAYAESHDQSIVGDKTIAFLLMDKEMYTGMSDLQPA
40
       SPTIDRGIALQKMIHFITMALGGDGYLNFMGNEFGHPEWIDFPREGNNWSYDKCRROW
       SLVDTDHLRYKYMNAFDQAMNALDERFSFLSSSKQIVSDMNDEEKVIVFERGDLVFVF
45
       NFHPKKTYEGYKVGCDLPGKYRVALDSDALVFGGHGRVGHDVDHFTSPEGVPGVPETN
       FNNRPNSFKVLSPPRTCVAYYRVDEAGAGRRLHAKAETGKTSPAESIDVKASRASSKE
                            DKEATAGGKKGWKFARQPSDQDTK"
            transit peptide 2..190
50
            mat_peptide
                            191..2467
                            /EC number="2.4.1.18"
                            /codon start=1
                            /product="branching enzyme-I precursor"
            polyA_signal
                            2734..2739
55
       BASE COUNT
                       719 A
                                585 C
                                          737 G
                                                  722 T
       ORIGIN
               1 GCTGTGCCTC GTGTCGCCCT CTTCCTCGCC GACTCCGCTT CCGCCGCCGC GGCGCTCTCG
              61 CTCGCATGCT GATCGGGCGG CACCGCCGGG GATCGCGGGT GGCGGCAATG TGCGCCTGAG
             121 TGTGTTGTCT GTCCAGTGCA AGGCTCGCCG GTCAGGGGTG CGGAAGGTCA AGAGCAAATT
60
             181 CGCCACTGCA GCTACTGTGC AAGAAGATAA AACTATGGCA ACTGCCAAAG GCGATGTCGA
             241 CCATCTCCCC ATATACGACC TGGACCCCAA GCTGGAGATA TTCAAGGACC ATTTCAGGTA
             301 CCGGATGAAA AGATTCCTAG AGCAGAAAGG ATCAATTGAA GAAAATGAGG GAAGTCTTGA
             361 ATCTTTTCT AAAGGCTATT TGAAATTTGG GATTAATACA AATGAGGATG GAACTGTATA
             421 TCGTGAATGG GCACCTGCTG CGCAGGAGGC AGAGCTTATT GGTGACTTCA ATGACTGGAA
65
             481 TGGTGCAAAC CATAAGATGG AGAAGGATAA ATTTGGTGTT TGGTCGATCA AAATTGACCA
             541 TGTCAAAGGG AAACCTGCCA TCCCTCACAA TTCCAAGGTT AAATTTCGCT TTCTACATGG
             601 TGGAGTATGG GTTGATCGTA TTCCAGCATT GATTCGTTAT GCGACTGTTG ATGCCTCTAA
```

	661	ATTTGGAGCT CCCTATGATG GTGTTCATTG GGATCCTCCT GCTTCTGAAA GGTACACATT
	721	TAAGCATCCT CGGCCTTCAA AGCCTGCTGC TCCACGTATC TATGAAGCCC ATGTAGGTAT
	841	GAGTGGTGAA AAGCCAGCAG TAAGCACATA TAGGGAATTT GCAGACAATG TGTTGCCACG CATACGAGCA AATAACTACA ACACAGTTCA GTTGATGGCA GTTATGGAGC ATTCGTACTA
5	901	TGCTTCTTTC GGGTACCATG TGACAAATTT CTTTGCGGTT AGCAGCAGAT CAGGCACACC
	961	AGAGGACCTC AAATATCTTG TTGATAAGGC ACACAGTTTG GGTTTGCGAG TTCTCATCCA
	1021	TGTTGTCCAT AGCCATGCAA GTAATAATGT CACAGATGGT TTAAATGGCT ATCATCTTCC
	1081	ACAAAGCACC CAAGAGTCCT ATTTTCATGC GGGAGATAGA GGTTATCATA AACTTTCCCA
10	1141	TAGTCGGCTG TTCAACTATG CTAACTGGGA GGTATTAAGG TTTCTTCTTT CTAACCTCAC
10	1201	ATATTGGTTG GATGAATTCA TGTTTGATGG CTTCCGATTT GATGGAGTTA CATCAATGCT
	1201	GTATCATCAC CATGGTATCA ATGTGGGGTT TACTGGAAAC TACCAGGAAT ATTTCAGTTT
	1381	GGACACAGCT GTGGATGCAG TTGTTTACAT GATGCTTGCA AACCATTTAA TGCACAAACT CTTGCCAGAA GCAACTGTTG TTGCTGAAGA TGTTTCAGGC ATGCCGGTCC TTTGCCGGCC
	1441	AGTTGATGAA GGTGGGGTTG GGTTTGACTA TCGCCTGGCA ATGGCTATCC CTGATAGATG
15	1501	GATTGACTAC CTGAAGAATA AAGATGACTC TGAGTGGTCG ATGGGTGAAA TAGCGCATAC
	1561	TTTGACTAAC AGGAGATATA CTGAAAAATG CATCGCATAT GCTGAGAGCC ATGATCAGTC
	1621	TATTGTTGGC GACAAAACTA TTGCATTTCT CCTGATGGAC AAGGAAATGT ACACTGGCAT
	1981	GTCAGACTTG CAGCCTGCTT CACCTACAAT TGATCGAGGG ATTGCACTCC AAAAGATGAT
20	1741	TCACTTCATC ACAATGGCCC TTGGAGGTGA TGGCTACTTG AATTTTATGG GAAATGAGTT
20	1801	TGGTCACCCA GAATGGATTG ACTTTCCAAG AGAAGGGAAC AACTGGAGCT ATGATAAATG
	1921	CAGACGACAG TGGAGCCTTG TGGACACTGA TCACTTGCGG TACAAGTACA TGAATGCGTT
	1981	TGACCAAGCG ATGAATGCGC TCGATGAGAG ATTTTCCTTC CTTTCGTCGT CAAAGCAGAT CGTCAGCGAC ATGAACGATG AGGAAAAGGT TATTGTCTTT GAACGTGGAG ATTTAGTTTT
	2041	TGTTTTCAAT TTCCATCCCA AGAAAACTTA CGAGGGCTAC AAAGTGGGAT GCGATTTGCC
25	2101	TGGGAAATAC AGAGTAGCCC TGGACTCTGA TGCTCTGGTC TTCGGTGAC ATGGAACACT
	2161	TGGCCACGAC GTGGATCACT TCACGTCGCC TGAAGGGGTG CCAGGGGTGC CCGAACGAA
	2221	CTTCAACAAC CGGCCGAACT CGTTCAAAGT CCTTTCTCCG CCCCGCACCT CTCTCGCTTA
	228T	TTACCGTGTA GACGAAGCAG GGGCTGGACG ACGTCTTCAC GCGAAAGCAG AGACAGGAAA
30	2341	GACGTCTCCA GCAGAGAGCA TCGACGTCAA AGCTTCCAGA GCTAGTAGCA AAGAAGACAA
J 0	2401	GGAGGCAACG GCTGGTGGCA AGAAGGGATG GAAGTTTGCG CGGCAGCCAT CCGATCAAGA
	2521	TACCAAATGA AGCCACGAGT CCTTGGTGAG GACTGGACTG
	2581	TGCAGGCGAC TGGTGTCTCA TCACCGAGCA GGCAGGCACT GCTTGTATAG CTTTTCTAGA
	2641	ATAATAATCA GGGATGGATG GATGGTGTGT ATTGGCTATC TGGCTAGACG TGCATGTGCC
35	2701	CAGTTTGTAT GTACAGGAGC AGTTCCCGTC CAGAATAAAA AAAAACTTGT TGGGGGGTTT
	2761	TTC
	//	
		TABLE 7
		Coding Sequence and Deduced Amino Acid Sequence for
40		Transit Peptide Region of the
		Soluble Starch Synthase I Maize Gene (153 bp)
		[SEQ ID NO:18 and SEQ ID NO:19]
	FILE	NAME : MSS1TRPT.DNA SEQUENCE : NORMAL 153 BP
	CODO	ON TABLE : UNIV.TCN
	0050	WIADLE : ONIV.ICA
45	SEQU	JENCE REGION: 1 - 153
	TRAN	SLATION REGION: 1 - 153
	*** DND	TRANSLATION ***
	DIA	IRANSLATION ***
	1 ATG G	CG ACG CCC TCG GCC GTG GGC GCC GCG TGC CTC CT
	1. M	A T P S A V G A A C L L A R 16
50	,	
30	49 GCC G	CC TGG CCG GCC GTC GGC GAC CGG GCG CGC CGG AGG CTC 96
	17 A	A W P A A V G D R A R P R R L 32
	97 CAG C	GC GTG CTG CGC CGC TGC GTC GCG GAG CTG AGC AGG GAG GGG 144
	33 Q	R V L R R R C V A E L S R E G 48
	-	
5 6		AT ATG 153
55	49 P	H M 51

GFP constructs:

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1. GFP only in pET-21a:

pEXS115 is digested with *Nde* I and *Xho* I and the 740 bp fragment containing the SGFP coding sequence is subcloned into the *Nde* I and *Xho* I sites of pET-21a (Novagen 601 Science Dr. Madison WI). (See FIG. 2b GFP-21a map.)

2. GFP subcloned in-frame at the 5' end of full-length mature WX:

The 740 bp Nde I fragment containing SGFP from pEXS114 is subcloned into the Nde I site of pEXSWX. (See FIG.3a GFP-FLWX map.)

3. GFP subcloned in-frame at the 5' end of N-terminally truncated WX:

WX truncated by 700 bp at N-terminus.

The 1 kb BamH I fragment encoding the C-terminus of WX from pEXSWX is subcloned into the Bgl II site of pEXS115. Then the entire SGFP-truncated WX fragment is subcloned into pET21a as a Nde I-HindIII fragment. (See FIG. 3b GFP-BamHIWX map.)

4. GFP subcloned in-frame at the 5' end of truncated WX: WX truncated by 100 bp at N-terminus.

The 740 bp *Nde* I-Nco I fragment containing SGFP from pEXS115 is subcloned into pEXSWX at the *Nde* I and *Nco* I sites. (See Fig. 4 GFP-NcoWX map.)

Example Three:

Plasmid Transformation into Bacteria:

Escherichia coli competent cell preparation:

- 1. Inoculate 2.5 ml LB media with a single colony of desired *E. coli* strain: selected strain was XLIBLUE DL2IDE3 from (Stratagene); included appropriate antibiotics. Grow at 37°C, 250 rpm overnight.
- Inoculate 100 ml of LB media with a 1:50 dilution of the overnight culture,
 including appropriate antibiotics. Grow at 37°C, 250 rpm until OD₆₀₀=0.3-0.5.
 - 3. Transfer culture to sterile centrifuge bottle and chill on ice for 15 minutes.

- 4. Centrifuge 5 minutes at 3,000x g (4°C).
- 5. Resuspend pellet in 8 ml ice-cold Transformation buffer. Incubate on ice for 15 minutes.
 - 6. Centrifuge 5 minutes at 3,000x g (4°C).
- 7. Resuspend pellet in 8 ml ice-cold Transformation buffer 2. Aliquot, flash-freeze in liquid nitrogen, and stored at -70°C.

	<u>Transformation</u>	on Buffer 1	Transformation Buffer 2					
	RbCl	1.2 g	MOPS (10 mM)	0.209 g				
	MnCl ₂ 4H ₂ O	0.99g	RbCl	0.12 g				
10	K-Acetate	0.294 g	CaCl ₂ 2H ₂ O	1.1 g				
	CaCl ₂ 2H ₂ O	0.15 g	Glycerol	15 g				
	Glycerol	15 g	dH ₂ O	100 ml				
	dH_2O	100 ml	pH to 6.8 with NaO	Н				
	pH to 5.8 with	th 0.2 M acetic acid	Filter sterilize					
15	Filter sterilize	2						

Escherichia coli transformation by rubidium chloride heat shock method: Hanahan, D. (1985) in DNA cloning: a practical approach (Glover, D.M. ed.), pp. 109-135, IRL Press.

- 1. Incubate 1-5 μ l of DNA on ice with 150 μ l E. coli competent cells for 30 minutes.
- 2. Heat shock at 42°C for 45 seconds.

20

- 3. Immediately place on ice for 2 minutes.
- 4. Add 600 μ l LB media and incubate at 37°C for 1 hour.

5. Plate on LB agar including the appropriate antibiotics.

This plasmid will express the hybrid polypeptide containing the green fluorescent protein within the bacteria.

Example Four:

5 Expression of Construct in E. coli:

- 1. Inoculate 3 ml LB with E. coli containing plasmid of interest. Include appropriate antibiotics. 37°C, 250 rpm, overnight.
- 2. Inoculate 100 ml LB with 2 ml of overnight culture. Include appropriate antibiotics. Grow at 37°C, 250 rpm.
- At OD₆₀₀ about 0.4-0.5, place at room temperature, 200 rpm.
 - 4. At OD₆₀₀ about 0.6-0.8, induce with 100 μ l 1M 1PTG. Final 1PTG concentration is 1 mM.
 - 5. Grow at room temperature, 200 rpm, 4-5 hours.
 - 6. Collect cells by centrifugation.

20

15 7. Flash freeze in liquid nitrogen and store at -70°C until use.

Cells can be resuspended in dH_2O and viewed under UV light ($\lambda_{max} = 395$ nm) for intrinsic fluorescence. Alternatively, the cells can be sonicated and an aliquot of the cell extract can be separated by SDS-PAGE and viewed under UV light to detect GFP fluorescence. When the protein employed is a green fluorescent protein, the presence of the protein in the lysed material can be evaluated under UV at 395 nm in a light box and the signature green glow can be identified.

Example Five:

Plasmid Extraction from Bacteria:

The following is one of many common alkaline lysis plasmid purification protocols useful in practicing this invention.

- Inoculate 100-200 ml LB media with a single colony of E. coli transformed with the one of the plasmids described above. Include appropriate antibiotics. Grow at 37°C, 250 rpm overnight.
 - 2. Centrifuge 10 minutes at 5,000x g (4°C).
- 3. Resuspend cells in 10 ml water, transfer to a 15 ml centrifuge tube, and repeat centrifugation.
 - 4. Resuspend pellet in 5 ml 0.1 M NaOH, 0.5% SDS. Incubate on ice for 10 minutes.
 - 5. Add 2.5 ml of 3 M sodium acetate (pH 5.2), invert gently, and incubate 10 minutes on ice.
 - 6. Centrifuge 5 minutes at 15,000-20,000x g (4°C).
- 15 7. Extract supernatant with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1).
 - 8. Centrifuge 10 minutes at 6,000-10,000x g (4°C).
 - 9. Transfer aqueous phase to clean tube and precipitate with 1 volume of isopropanol.
 - 10. Centrifuge 15 minutes at 12,000x g (4°C).
- 20 11. Dissolve pellet in 0.5 ml TE, add 20 μ l of 10 mg/ml Rnase, and incubate 1 hour at 37°C.

- 12. Extract twice with phenol:chloroform:isoamyl alcohol (25:24:1).
- 13. Extract once with chloroform.
- 14. Precipitate aqueous phase with 1 volume of isopropanol and 0.1 volume of 3 M sodium acetate.
- 5 15. Wash pellet once with 70% ethanol.
 - 16. Dry pellet in SpeedVac and resuspend pellet in TE.

This plasmid can then be inserted into other hosts.

TABLE 8

1626

DNA Sequence and Deduced Amino Acid Sequence of

Starch Synthase Coding Region from pEXS52 [SEQ ID NO:20; SEQ ID NO:21]

1 -

FILE NAME : MSS1DELN.DNA SEQUENCE : NORMAL 1626 BP

CODON TABLE : UNIV.TCN

SEQUENCE REGION : 1 - 1626

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

TRANSLATION REGION :

					CTG Leu												48	8
20					TCC Ser												90	6
					GTG Val												14	4
25					TTT Phe												193	2
30		_			GAT Asp 120												24	0
	CGT	GGT	CAC	CGT	GTG	ATG	GTT	GTA	ATG	ccc	AGA	TAT	TTA	AAT	GGT	ACC	28	8

CGT GGT CAC CGT GTG ATG GTT GTA ATG CCC AGA TAT TTA AAT GGT ACC Arg Gly His Arg Val Met Val Val Met Pro Arg Tyr Leu Asn Gly Thr

135	140	145
-----	-----	-----

	TCC Ser	GAT Asp	AAG Lys 150	TAA Asn	TAT Tyr	GCA Ala	Asn	GCA Ala 155	TTT Phe	TAC Tyr	ACA Thr	Giu	AAA Lys 160	CAC His	ATT Ile	CGG Arg	336
5	ATT Ile	CCA Pro 165		TTT Phe	GGC Gly	GGT Gly	CAA	ሮልሞ	GAA Glu	GTT Val	ACC Thr	TTC Phe 175	TTC Phe	CAT His	GAG Glu	TAT Tyr	384
10	AGA Arg 180		TCA Ser	GTT Val	GAC Asp	TGG Trp 185	GTG Val	TTT Phe	GTT Val	GAT Asp	CAT His 190	CCC Pro	TCA Ser	TAT Tyr	CAC His	AGA Arg 195	432
	CCT Pro	GGA Gly	AAT Asn	TTA Leu	TAT Tyr 200	GGA Gly	GAT Asp	AAG Lys	TTT Phe	GGT Gly 205	GCT Ala	TTT Phe	GGT Gly	GAT Asp	AAT Asn 210	CAG Gln	480
15	TTC Phe	AGA Arg	TAC Tyr	ACA Thr 215	CTC Leu	CTT Leu	TGC Cys	TAT Tyr	GCT Ala 220	GCA Ala	TGT Cys	GAG Glu	GCT Ala	CCT Pro 225	TTG Leu	ATC Ile	528
	CTT Leu	GAA Glu	TTG Leu 230	Gly	GGA Gly	TAT Tyr	ATT Ile	TAT Tyr 235	GGA Gly	CAG Gln	AAT Asn	TGC Cys	ATG Met 240	TTT Phe	GTT Val	GTC Val	576
20	AAT Asn	GAT Asp 245	Trp	CAT His	GCC Ala	AGT Ser	CTA Leu 250	GTG Val	CCA Pro	GTC Val	CTT Leu	CTT Leu 255	MIG	GCA Ala	AAA Lys	TAT Tyr	624
25	AGA Arg 260	Pro	TAT Tyr	GGT Gly	GTT Val	TAT Tyr 265	AAA Lys	GAC Asp	TCC	CGC Arg	AGC Ser 270	TIF	CTT Leu	GTA Val	ATA Ile	CAT His 275	672
	AAT Asn	TTA Lev	A GCA 1 Ala	CAT His	CAG Gln 280	Gly	GTA Val	GAG Glu	CCT Pro	GCA Ala 285	Ser	ACA Thr	TAT Tyr	CCT Pro	GAC Asp 290	CTT Leu	720
30	GGG Gly	TTC	G CCA	CCI Pro 295	Glu	TGG Trp	TAT	GGA Gly	GCT Ala 300	Leu	GAG Glu	TGG Trp	GTA Val	TTC Phe 305		GAA Glu	768
	TG0 Tr	1 A 1 :	a Arc	r Arc	7 His	GCC Ala	Leu	Ast) Lys	GIY	GIO	HTC	A GTT a Val 320	. ASI	TTI Phe	TTG Leu	816
35	AA <i>l</i> Lys	A GG' 5 Gl; 32	y Ala	A GT1 a Val	r GTO	ACA L Thr	GCA Ala 330	Asp	y Ard	ATC ; Ile	GTC Val	3 ACT L Thi 335	va.	C AGT L Ser	AAC Lys	G GGT	864
40	TA: Ty: 340	r Se	G TGG	G GAG p Gl	G GTO	C ACA L Thr 345	Thr	GCT Ala	r GAZ a Glu	A GGT	GGA Gly 350	y Gii	G GGG	C CTO y Lev	C AAT	r GAG n Glu 355	912
	CT(Le	C TT u Le	A AG u Se	C TC	C AGA	ā rā	G AG1	GT Va	A TTZ l Lev	A AAG 1 Asi 369	1 613	A AT	r GT: e Va	A AA' l Asi	r GG n Gl 37	A ATT y Ile O	960
45	GA As	C AT p Il	T AA e As	T GA n As 37	p Tr	G AAG p Ası	C CC	r GC	C AC a Th	r As	C AA	A TG s Cy	T AT s Il	C CC e Pr 38	o cy	T CAT s His	1008
	TA Ty	T TC r Se	T GT r Va 39	l As	T GA p As	C CTO	C TC	r GG r Gl 39	Ă ŗĂ	G GC s Al	C AA a Ly	A TG s Cy	T AA s Ly 40	5 61	T GC y Al	A TTG a Leu	1056

	CAG AA Gln Ly 40	's Glu	CTG Leu	GGT Gly	TTA Leu	CCT Pro 410	ATA Ile	AGG Arg	CCT Pro	GAT Asp	GTT Val 415	CCT Pro	CTG Leu	ATT Ile	GGC Gly	1104
5	TTT AT Phe Il 420															1152
	ATC AT Ile Il															1200
10	TCT GG Ser Gl	T GAC Y Asp	CCA Pro 455	GAG Glu	CTT Leu	GAA Glu	GAT Asp	TGG Trp 460	ATG Met	AGA Arg	TCT Ser	ACA Thr	GAG Glu 465	TCG Ser	ATC Ile	1248
15	TTC AA Phe Ly															1296
	CAC CG His Ar 48	g Ile														1344
20	GAA CC Glu Pr 500															1392
	CCT GT Pro Va															1440
25	AAC CC Asn Pr	T TTC O Phe	GGT Gly 535	GAG Glu	AAT Asn	GGA Gly	GAG Glu	CAG Gln 540	GGT Gly	ACA Thr	GGG Gly	TGG Trp	GCA Ala 545	TTC Phe	GCA Ala	1488
30	CCC CT Pro Le	A ACC u Thr 550	ACA Thr	GAA Glu	AAC Asn	ATG Met	TTT Phe 555	GTG Val	GAC Asp	ATT Ile	GCG Ala	AAC Asn 560	TGC Cys	AAT Asn	ATC Ile	1536
	TAC AT Tyr Il 56	e Gln	GGA Gly	ACA Thr	CAA Gln	GTC Val 570	CTC Leu	CTG Leu	GGA Gly	AGG Arg	GCT Ala 575	AAT Asn	GAA Glu	GCG Ala	AGG Arg	1584
35	CAT GT His Va 580	C AAA l Lys	AGA Arg	CTT Leu	CAC His 585	GTG Val	GGA Gly	CCA Pro	TGC Cys	CGC Arg 590	TGA *					1620
	(2) IN	FORMA	NOI	FOR	SEQ	ID N	10:21	. :								
40		(i) S	(A)	LEN TYP	CHAFIGTH:	: 540 imino) ami o aci	.no a		5						
		(ii) }	OLEC	CULE	TYPE	: pr	otei	.n								
		(xi) 5										~ 1		-1	, ,	
45	Cys Va 1	T WIG	GIU	Leu 5	ser	Arg	GIU	Asp	10	GTÅ	Leu	GIU	rro	GIu 15	GTÅ	
	Ile Al	a Glu	Gly 20	Ser	Ile	Asp	Asn	Thr 25	Val	Val	Val	Ala	Ser 30	Glu	Gln	
	Asp Se	r Glu 35	Ile	Val	Val	Gly	Lys 40	Glu	Gln	Ala	Arg	Ala 45	Lys	Val	Thr	

	Gln	Ser	Ile	Val	Phe	Val		Gly	Glu	Ala	Ser		Tyr	Ala	Lys	Ser
	Gly	50 Gly	Lou	Gly	Aen	Va 1	55 Cvs	Glv	Ser	ī.eu	Pro	60 Val	Ala	Leu	Δla	Ala
	65	GIY	Leu	GIY	vaħ	70	Cys	Gly	Jer	Deu	75	vai	nia	Deu	ura	80
5	Arg	Gly	His	Arg	Val 85	Met	Val	Val	Met	Pro 90	Arg	Tyr	Leu	Asn	Gly 95	Thr
	Ser	Asp	ГÀа	Asn 100	Tyr	Ala	Asn	Ala	Phe 105	Tyr	Thr	Glu	Lys	His 110	Ile	Arg
10	Ile	Pro	Cys 115	Phe	Gly	Gly	Glu	His 120	Glu	Val	Thr	Phe	Phe 125	His	Glu	Tyr
	Arg	Asp 130	Ser	Val	Asp	Trp	Val 135	Phe	Val	Asp	His	Pro 140	Ser	Tyr	His	Arg
	Pro 145	Gly	Asn	Leu	Tyr	Gly 150	Asp	ГÀа	Phe	Gly	Ala 155	Phe	Gly	Asp	Asn	Gln 160
15	Phe	Arg	Tyr	Thr	Leu 165	Leu	Cys	Tyr	Ala	Ala 170	Cys	Glu	Ala	Pro	Leu 175	Ile
	Leu	Glu	Leu	Gly 180	Gly	Tyr	Ile	Tyr	Gly 185	Gln	Asn	Cys	Met	Phe 190	Val	Val
20	Asn	Asp	Trp 195	His	Ala	Ser	Leu	Val 200	Pro	Val	Leu	Leu	Ala 205	Ala	Lys	Tyr
	Arg	Pro 210	Tyr	Gly	Val	Tyr	Lys 215	Asp	Ser	Arg	Ser	Ile 220	Leu	Val	Ile	His
	Asn 225	Leu	Ala	His	Gln	Gly 230	Val	Glu	Pro	Ala	Ser 235	Thr	Tyr	Pro	Asp	Leu 240
25	Gly	Leu	Pro	Pro	Glu 245	Trp	Tyr	Gly	Ala	Leu 250	Glu	Trp	Val	Phe	Pro 255	Glu
	Trp	Ala	Arg	Arg 260	His	Ala	Leu	qzƙ	Lys 265	Gly	Glu	Ala	Val	Asn 270	Phe	Leu
30	Lys	Gly	Ala 275	Val	Val	Thr	Ala	Asp 280	Arg	Ile	Val	Thr	Val 285	Ser	Lys	Gly
	Tyr	Ser 290	Trp	Glu	Val	Thr	Thr 295	Ala	Glu	Gly	Gly	Gln 300	Gly	Leu	Asn	Glu
	Leu 305	Leu	Ser	Ser	Arg	Lys 310	Ser	Val	Leu	Asn	Gly 315	Ile	Val	Asn	Gly	Ile 320
35	Asp	Ile	Asn	Asp	Trp 325	Asn	Pro	Ala	Thr	Asp 330	Lys	Cys	Ile	Pro	Cys 335	His
	Tyr	Ser	Val	Asp 340	Asp	Leu	Ser	Gly	Lys 345	Ala	Lys	Cys	Lys	Gly 350	Ala	Leu
40	Gln	Lys	Glu 355	Leu	Gly	Leu	Pro	Ile 360	Arg	Pro	Asp	Val	Pro 365	Leu	Ile	Gly
	Phe	Ile 370	Gly	Arg	Leu	Asp	Tyr 375	Gln	Lys	Gly	Ile	Asp 380	Leu	Ile	Gln	Leu
	Ile 385	Ile	Pro	Asp	Leu	Met 390	Arg	Glu	Asp	Val	Gln 395	Phe	Val	Met	Leu	Gly 400
45	Ser	Gly	Asp	Pro	Glu 405	Leu	Glu	Asp	Trp	Met 410	Arg	Ser	Thr	Glu	Ser 415	Ile

Phe Lys Asp Lys Phe Arg Gly Trp Val Gly Phe Ser Val Pro Val Ser 420

His Arg Ile Thr Ala Gly Cys Asp Ile Leu Leu Met Pro Ser Arg Phe 435

Glu Pro Cys Gly Leu Asn Gln Leu Tyr Ala Met Gln Tyr Gly Thr Val 450

Pro Val Val His Ala Thr Gly Gly Leu Arg Asp Thr Val Glu Asn Phe 465

Asn Pro Phe Gly Glu Asn Gly Glu Gln Gly Thr Gly Trp Ala Phe Ala 485

Pro Leu Thr Thr Glu Asn Met Phe Val Asp Ile Ala Asn Cys Asn Ile 500

Tyr Ile Gln Gly Thr Gln Val Leu Leu Gly Arg Ala Asn Glu Ala Arg 515

His Val Lys Arg Leu His Val Gly Pro Cys Arg * 540

Example Six:

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This experiment employs a plasmid having a maize promoter, a maize transit peptide, a starch-encapsulating region from the starch synthase I gene, and a ligated gene fragment attached thereto. The plasmid shown in FIG. 6 contains the DNA sequence listed in Table 8.

Plasmid pEXS52 was constructed according to the following protocol:

Materials used to construct transgenic plasmids are as follows:

Plasmid pBluescript SK-

Plasmid pMF6 (contain nos3' terminator)

25 Plasmid pHKH1 (contain maize adh1 intron)

Plasmid MstsI(6-4) (contain maize stsI transit peptide, use as a template for PCT stsI transit peptide out)

Plasmid MstsIII in pBluescript SK-

Primers EXS29 (GTGGATCCATGGCGACGCCCTCGGCCGTGG) [SEQ ID NO:22]

30 EXS35 (CTGAATTCCATATGGGGCCCCTCCCTGCTCAGCTC) [SEQ ID NO:23] both used for PCT stsI transit peptide

Primers EXS31 (CTCTGAGCTCAAGCTTGCTACTTTCTTTCCTTAATG) [SEQ ID NO:24]

EXS32 (GTCTCCGCGGTGGTGTCCTTGCTTCCTAG) [SEQ ID NO:25] both used for PCR maize 10KD zein promoter (Journal: Gene 71:359-370 [1988]) Maize A632 genomic DNA (used as a template for PCR maize 10KD zein promoter).

Step 1: Clone maize 10KD zein promoter in pBluescriptSK-(named as pEXS10zp).

PCR 1.1Kb maize 10KD zein promoter primers: EXS31, EXS32

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template: maize A632 genomic DNA

2. Clone 1.1Kb maize, 10KD zein promoter PCR product into pBluescript SK-plasmid at SacI and SacII site (See FIG. 7).

10 Step 2: Delete NdeI site in pEXS10zp (named as pEXS10zp-NdeI).

NdeI is removed by fill in and blunt end ligation from maize 10KD zein promoter in pBluescriptSK.

Step 3: Clone maize adh1 intron in pBluescriptSK- (named as pEXSadh1).

Maize adhl intron is released from plasmid pHKHl at XbaI and BamHl sites. Maize adhl intron (XbaI/BamHl fragment) is cloned into pBluescriptSK- at XbaI and BamHl sites (see FIG. 7).

Step 4: Clone maize 10KD zein promoter and maize adh1 intron into pBluescriptSK-(named as pEXS10zp-adh1).

Maize 10KD zein promoter is released from plasmid pEXS 10zp-NdeI at SacI and SacII sites. Maize 10KD zein promoter (SacI/SacII fragment) is cloned into plasmid pEXSadh1 (contain maize adh1 intron) at SacI and SacII sites (see FIG. 7).

Step 5: Clone maize nos3' terminator into plasmid pEXSadh1 (named as pEXSadh1-nos3').

Maize nos3' terminator is released from plasmid pMF6 at EcoRI and HindIII sites.

Maize nos3' terminator (EcoRI/HindIII fragment) is cloned into plasmid pEXSadh1 at

EcoRI and HindIII (see FIG. 7).

Step 6: Clone maize nos3' terminator into plasmid pEXS10zp-adh1 (named as pEXS10zp-adh1-nos3').

Maize nos3' terminator is released from plasmid pEXSadh1-nos3' at EcoRI and ApaI sites. Maize nos3' terminator (EcoRI/ApaI fragment) is cloned into plasmid pEXS10zp-adh1 at EcoRI and ApaI sites (see FIG. 7).

Step 7: Clone maize STSI transit peptide into plasmid pEXS10zp-adh1-nos3' (named as pEXS33).

 PCR 150bp maize STSI transit peptide primer: EXS29, EXS35

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template: MSTSI(6-4) plasmid

2. Clone 150bp maize STSI transit peptide PCR product into plasmid pEXS10zp-adh1-nos3' at EcoRI and BamHI sites (see FIG. 7).

Step 8: Site-directed mutagenesis on maize STSI transit peptide in pEXS33 (named as pEXS33(m)).

There is a mutation (stop codon) on maize STSI transit peptide in plasmid pEXS33.

Site-directed mutagenesis is carried out to change stop codon to non-stop codon. New plasmid (containing maize 10KD zein promoter, maize STSI transit peptide, maize adh1 intron, maize nos3' terminator) is named as pEXS33(m).

Step 9: NotI site in pEXS33(m) deleted (named as pEXS50).

NotI site is removed from pEXS33 by NotI fillin, blunt end ligation to form pEXS50 (see FIG. 8).

Step 10: Maize adh1 intron deleted in pEXS33(m) (named as pEXS60).

Maize adh1 intron is removed by NotI/BamHI digestion, filled in with Klenow fragment, blunt end ligation to form pEXS60 (see FIG. 9).

Step 11: Clone maize STSIII into pEXS50, pEXS60.

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Maize STSIII is released from plasmid maize STSIII in pBluescript SK- at NdeI and EcoRI sites. Maize STSIII (NdeI-EcoRI fragment) is cloned into pEXS50, pEXS60 separately, named as pEXS51, pEXS61 (see FIGS. 8 and 9, respectively).

Step 12: Clone the gene in Table 8 into pEXS51 at NdeI/NotI site to form pEXS52.

Other similar plasmids can be made by cloning other genes (STSI, II, WX, glgA, glgB, glgC, BEI, BEII, etc.) into pEXS51, pEXS61 at NdeI/NotI site.

Plasmid EXS52 was transformed into rice. The regenerated rice plants transformed with pEXS52 were marked and placed in a magenta box.

Two siblings of each line were chosen from the magenta box and transferred into 2.5 inch pots filled with soil mix (topsoil mixed with peat-vermiculite 50/50). The pots were placed in an aquarium (fish tank) with half an inch of water. The top was covered to maintain high humidity (some holes were made to help heat escape). A thermometer monitored the temperature. The fish tank was placed under fluorescent lights. No fertilizer was used on the plants in the first week. Light period was 6 a.m.-8 p.m., minimum 14 hours light. Temperature was minimum 68°F at night, 80°-90°F during the day. A heating mat was used under the fish tank to help root growth when necessary. The plants stayed in the

above condition for a week. (Note: the seedlings began to grow tall because of low light intensity.)

After the first week, the top of the aquarium was opened and rice transformants were transferred to growth chambers for three weeks with high humidity and high light intensity.

Alternatively, water mix in the greenhouse can be used to maintain high humidity. The plants grew for three weeks. Then the plants were transferred to 6-inch pots (minimum 5-inch pots) with soil mix (topsoil and peat-Vet, 50/50). The pots were in a tray filled with half an inch of water. 15-16-17 (N-K-P) was used to fertilize the plants (250 ppm) once a week or according to the plants' needs by their appearances. The plants remained in 14 hours

The plants formed rice grains and the rice grains were harvested. These harvested seeds can have the starch extracted and analyzed for the presence of the ligated amino acids C, V, A, E, L, S, R, E [SEQ ID NO:27] in the starch within the seed.

light (minimum) 6 a.m.-8 p.m. high light intensity, temperature 85°-90°/70°F day/night.

Example Seven:

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15 SER Vector for Plants:

The plasmid shown in Figure 6 is adapted for use in monocots, i.e., maize. Plasmid pEXS52 (FIG. 6) has a promoter, a transit peptide (from maize), and a ligated gene fragment (TGC GTC GCG GAG CTG AGC AGG GAG) [SEQ ID NO:26] which encodes the amino acid sequence C V A E L S R E [SEQ ID NO:27].

This gene fragment naturally occurs close to the N-terminal end of the maize soluble starch synthase (MSTSI) gene. As is shown in TABLE 8, at about amino acid 292 the SER from the starch synthase begins. This vector is preferably transformed into a maize host. The transit peptide is adapted for maize so this is the preferred host. Clearly the transit peptide and the promoter, if necessary, can be altered to be appropriate for the host plant desired. After transformation by "whiskers" technology (U.S. Patent Nos. 5,302,523 and 5,464,765), the transformed host cells are regenerated by methods known in the art, the

transformant is pollinated, and the resultant kernels can be collected and analyzed for the presence of the peptide in the starch and the starch granule.

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This plasmid may be transformed into other cereals such as rice, wheat, barley, oats, sorghum, or millet with little to no modification of the plasmid. The promoter may be the waxy gene promoter whose sequence has been published, or other zein promoters known to the art.

Additionally these plasmids, without undue experimentation, may be transformed into dicots such as potatoes, sweet potato, taro, yam, lotus cassava, peanuts, peas, soybean, beans, or chickpeas. The promoter may be selected to target the starch-storage area of particular dicots or tubers, for example the patatin promoter may be used for potato tubers.

Various methods of transforming monocots and dicots are known in the industry and the method of transforming the genes is not critical to the present invention. The plasmid can be introduced into *Agrobacterium tumefaciens* by the freeze-thaw method of An et al. (1988) Binary Vectors, in Plant Molecular Biology Manual A3, S.B. Gelvin and R.A. Schilperoot, eds. (Dordrecht, The Netherlands: Kluwer Academic Publishers), pp. 1-19. Preparation of *Agrobacterium* inoculum carrying the construct and inoculation of plant material, regeneration of shoots, and rooting of shoots are described in Edwards et al., "Biochemical and molecular characterization of a novel starch synthase from potatoes," Plant J. 8, 283-294 (1995).

A number of encapsulating regions are present in a number of different genes.

Although it is preferred that the protein be encapsulated within the starch granule (granule encapsulation), encapsulation within non-granule starch is also encompassed within the scope of the present invention in the term "encapsulation." The following types of genes are useful for this purpose.

Use of Starch-Encapsulating Regions of Glycogen Synthase:

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E. coli glycogen synthase is not a large protein: the structural gene is 1431 base pairs in length, specifying a protein of 477 amino acids with an estimated molecular weight of 49,000. It is known that problems of codon usage can occur with bacterial genes inserted into plant genomes but this is generally not so great with E. coli genes as with those from other bacteria such as those from Bacillus. Glycogen synthase from E. coli has a codon usage profile much in common with maize genes but it is preferred to alter, by known procedures, the sequence at the translation start point to be more compatible with a plant consensus sequence:

glgA G A T A A T G C A G [SEQ ID NO:31] cons A A C A A T G G C T [SEQ ID NO:32]

Use of Starch-Encapsulating Regions of Soluble Starch Synthase:

cDNA clones of plant-soluble starch synthases are described in the background section above and can be used in the present invention. The genes for any such SSTS protein may be used in constructs according to this invention.

Use of Starch-Encapsulating Regions of Branching Enzyme:

cDNA clones of plant, bacterial and animal branching enzymes are described in the background section above can be used in the present invention. Branching enzyme [1,4Dglucan: 1,4Dglucan 6D(1,4Dglucano) transferase (E.C. 2.4.1.18)] converts amylose to amylopectin, (a segment of a 1,4Dglucan chain is transferred to a primary hydroxyl group in a similar glucan chain) sometimes called Q-enzyme.

The sequence of maize branching enzyme I was investigated by Baba et al. (1991) BBRC, 181:87-94. Starch branching enzyme II from maize endosperm was investigated by

Fisher et al. (1993) Plant Physiol, 102:1045-1046. The BE gene construct may require the presence of an amyloplast transit peptide to ensure its correct localization in the amyloplast. The genes for any such branching enzyme of GBSTS protein may be used in constructs according to this invention.

5 Use of Starch-Binding Domains of Granule-Bound Starch Synthase:

The use of cDNA clones of plant granule-bound starch synthases are described in Shure et al. (1983) Cell 35:225-233, and Visser et al. (1989) Plant Sci. 64(2):185-192. Visser et al. have also described the inhibition of the expression of the gene for granule-bound starch synthase in potato by antisense constructs (1991) Mol. Gen. Genetic 225(2):289-296; (1994) The Plant Cell 6:43-52.) Shimada et al. show antisense in rice (1993) Theor. Appl. Genet. 86:665-672. Van der Leij et al. show restoration of amylose synthesis in low-amylose potato following transformation with the wild-type waxy potato gene (1991) Theor. Appl. Genet. 82:289-295.

The amino acid sequences and nucleotide sequences of granule starch synthases from, for example, maize, rice, wheat, potato, cassava, peas or barley are well known. The genes for any such GBSTS protein may be used in constructs according to this invention.

Construction of Plant Transformation Vectors:

Plant transformation vectors for use in the method of the invention may be constructed using standard techniques

Use of Transit Peptide Sequences:

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Some gene constructs require the presence of an amyloplast transit peptide to ensure correct localization in the amyloplast. It is believed that chloroplast transit peptides have similar sequences (Heijne et al. describe a database of chloroplast transit peptides in (1991) Plant Mol. Biol. Reporter, 9(2):104-126). Other transit peptides useful in this invention are those of ADPG pyrophosphorylase (1991) Plant Mol. Biol. Reporter, 9:104-126), small subunit RUBISCO, acetolactate synthase, glyceraldehyde3Pdehydrogenase and nitrite reductase.

The consensus sequence of the transit peptide of small subunit RUBISCO from many genotypes has the sequence:

MASSMLSSAAVATRTNPAQASM VAPFTGLKSAAFPVSRKQNLDI TSIASNGGRVQC [SEQ ID NO:33]

The corn small subunit RUBISCO has the sequence:

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MAPTVMMASSATATRTNPAQAS AVAPFQGLKSTASLPVARRSSR SLGNVASNGGRIRC [SEQ ID NO:34]

The transit peptide of leaf glyceraldehyde3Pdehydrogenase from corn has the sequence:

MAQILAPSTQWQMRITKTSPCA TPITSKMWSSLVMKQTKKVAHS
AKFRVMAVNSENGT [SEQ ID NO:35]

The transit peptide sequence of corn endosperm-bound starch synthase has the sequence:

MAALATSQLVATRAGHGVPDASTFRRGAAQGLRGARASAAADTLSMRTSARAAPRHQ QQARRGGRFPFPSLVVC [SEQ ID NO:36]

The transit peptide sequence of corn endosperm soluble starch synthase has the sequence:

MATPSAVGAACLLLARXAWPAAVGDRARPRRLQRVLRRR [SEQ ID NO:37]

Engineering New Amino Acids or Peptides into Starch-Encapsulating Proteins:

The starch-binding proteins used in this invention may be modified by methods known to those skilled in the art to incorporate new amino acid combinations. For example,

sequences of starch-binding proteins may be modified to express higher-than-normal levels of lysine, methionine or tryptophan. Such levels can be usefully elevated above natural levels and such proteins provide nutritional enhancement in crops such as cereals.

In addition to altering amino acid balance, it is possible to engineer the starch-binding proteins so that valuable peptides can be incorporated into the starch-binding protein. Attaching the payload polypeptide to the starch-binding protein at the N-terminal end of the protein provides a known means of adding peptide fragments and still maintaining starch-binding capacity. Further improvements can be made by incorporating specific protease cleavage sites into the site of attachment of the payload polypeptide to the starch-encapsulating region. It is well known to those skilled in the art that proteases have preferred specificities for different amino-acid linkages. Such specificities can be used to provide a vehicle for delivery of valuable peptides to different regions of the digestive tract of animals and man.

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In yet another embodiment of this invention, the payload polypeptide can be released following purification and processing of the starch granules. Using amylolysis and/or gelatinization procedures it is known that the proteins bound to the starch granule can be released or become available for proteolysis. Thus recovery of commercial quantities of proteins and peptides from the starch granule matrix becomes possible.

In yet another embodiment of the invention it is possible to process the starch granules in a variety of different ways in order to provide a means of altering the digestibility of the starch. Using this methodology it is possible to change the bioavailablility of the proteins, peptides or amino acids entrapped within the starch granules.

Although the foregoing invention has been described in detail by way of illustration and example for purposes of clarity and understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Keeling, Peter
 Guan, Hanping
- (ii) TITLE OF INVENTION: Starch Encapsulation
- (iii) NUMBER OF SEQUENCES: 37
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Greenlee, Winner and Sullivan, P.C.
 - (B) STREET: 5370 Manhattan Circle
 - (C) CITY: Boulder
 - (D) STATE: CO
 - (E) COUNTRY: US
 - (F) ZIP: 80303
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: US
 - (B) FILING DATE: 30-SEP-1997
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 60/026,855
 - (B) FILING DATE: 30-SEP-1996
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Winner, Ellen P
 - (B) REGISTRATION NUMBER: 28,547
 - (C) REFERENCE/DOCKET NUMBER: 89-97
 - (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (303) 499-8080
 - (B) TELEFAX: (303) 499-8089
- (2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:	•	:
(A) LENGTH: 31 base pairs		
(B) TYPE: nucleic acid		
(C) STRANDEDNESS: single		
(D) TOPOLOGY: linear		
(b) 10102001. 12022		
(ii) MOLECULE TYPE: other nucleic acid		
(A) DESCRIPTION: /desc = "Oligonucleotide"		
(iii) HYPOTHETICAL: NO		
•		
-		
	٠.	
(with enoughide Decomposion, SEO ID NO.1.		
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:		
GACTAGTCAT ATGGTGAGCA AGGGCGAGGA G		31
(2) INFORMATION FOR SEQ ID NO:2:		
(i) SEQUENCE CHARACTERISTICS:		
(A) LENGTH: 36 base pairs		
(B) TYPE: nucleic acid		
(C) STRANDEDNESS: single		
(D) TOPOLOGY: linear		
•		
(ii) MOLECULE TYPE: other nucleic acid		
(A) DESCRIPTION: /desc = "Oligonucleotide"		
(iii) HYPOTHETICAL: NO		
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:		
(11) 002001100 00001101111111111111111111		
CTAGATCTTC ATATGCTTGT ACAGCTCGTC CATGCC		36
(2) INFORMATION FOR SEQ ID NO:3:		
(i) SEQUENCE CHARACTERISTICS:		
(A) LENGTH: 39 base pairs		
(B) TYPE: nucleic acid		

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(A) DESCRIPTION: /desc = "Oligonucleotide"	
(iii) HYPOTHETICAL: NO	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:	
CTAGATCTTG GCCATGGCCT TGTACAGCTC GTCCATGCC	39
(2) INFORMATION FOR SEQ ID NO:4:	
(i) SEQUENCE CHARACTERISTICS:	
(A) LENGTH: 4800 base pairs	
(B) TYPE: nucleic acid	
(C) STRANDEDNESS: double	
(D) TOPOLOGY: not relevant	
(ii) MOLECULE TYPE: DNA (genomic)	
(iii) HYPOTHETICAL: NO	
(vi) ORIGINAL SOURCE:	
(A) ORGANISM: Zea mays	
(ix) FEATURE:	
(A) NAME/KEY: CDS	
(B) LOCATION: join(14491553, 16851765, 18601958, 2055	
2144, 22262289, 24132513, 26512760, 2858	
3101, 32123394, 34903681, 37933879, 3977	
4105, 42274343)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:	-
CAGCGACCTA TTACACAGCC CGCTCGGGCC CGCGACGTCG GGACACATCT TCTTCCCCCT	60
TTTGGTGAAG CTCTGCTCGC AGCTGTCCGG CTCCTTGGAC GTTCGTGTGG CAGATTCATC 1	20
TGTTGTCTCG TCTCCTGTGC TTCCTGGGTA GCTTGTGTAG TGGAGCTGAC ATGGTCTGAG 1	80
CAGGCTTAAA ATTTGCTCGT AGACGAGGAG TACCAGCACA GCACGTTGCG GATTTCTCTG 2	40

(ii) MOLECULE TYPE: other nucleic acid

CCTGTGAAGT GCAACGTCTA GGATTGTCAC ACGCCTTGGT CGCGTCGCGT	300
CGATGCGGTG GTGAGCAGAG CAGCAACAGC TGGGCGGCCC AACGTTGGCT TCCGTGTCTT	360
CGTCGTACGT ACGCGCGCGC CGGGGACACG CAGCAGAGAG CGGAGAGCGA GCCGTGCACG	420
GGGAGGTGGT GTGGAAGTGG AGCCGCGCGC CCGGCCGCCC GCGCCCGGTG GGCAACCCAA	480
AAGTACCCAC GACAAGCGAA GGCGCCAAAG CGATCCAAGC TCCGGAACGC AACAGCATGC	540
GTCGCGTCGG AGAGCCAGCC ACAAGCAGCC GAGAACCGAA CCGGTGGGCG ACGCGTCATG	600
GGACGGACGC GGGCGACGCT TCCAAACGGG CCACGTACGC CGGCGTGTGC GTGCGTGCAG	660
ACGACAAGCC AAGGCGAGGC AGCCCCCGAT CGGGAAAGCG TTTTGGGCGC GAGCGCTGGC	720
GTGCGGGTCA GTCGCTGGTG CGCAGTGCCG GGGGGGAACGG GTATCGTGGG GGGCGCGGGC	780
GGAGGAGAGC GTGGCGAGGGG CCGAGAGCAG CGCGCCGCCG GGTCACGCAA CGCGCCCCAC	840
GTACTGCCCT CCCCCTCCGC GCGCGCTAGA AATACCGAGG CCTGGACCGG GGGGGGGCCC	900
CGTCACATCC ATCCATCGAC CGATCGATCG CCACAGCCAA CACCACCCGC CGAGGCGACG	960
CGACAGCCGC CAGGAGGAAG GAATAAACTC ACTGCCAGCC AGTGAAGGGG GAGAAGTGTA	1020
CTGCTCCGTC GACCAGTGCG CGCACCGCCC GGCAGGGCTG CTCATCTCGT CGACGACCAG	1080
GTTCTGTTCC GTTCCGATCC GATCCGATCC TGTCCTTGAG TTTCGTCCAG ATCCTGGCGC	1140
GTATCTGCGT GTTTGATGAT CCAGGTTCTT CGAACCTAAA TCTGTCCGTG CACACGTCTT	1200
TTCTCTCTCT CCTACGCAGT GGATTAATCG GCATGGCGGC TCTGGCCACG TCGCAGCTCG	1260
TCGCAACGCG CGCCGGCCTG GGCGTCCCGG ACGCGTCCAC GTTCCGCCGC GGCGCCGCGC	1320
AGGGCCTGAG GGGGGCCCGG GCGTCGGCGG CGGCGGACAC GCTCAGCATG CGGACCAGCG	1380
CGCGCGCGCG GCCCAGGCAC CAGCAGCAGG CGCGCCGCG GGGCAGGTTC CCGTCGCTCG	1440
TCGTGTGC GCC AGC GCC GGC ATG AAC GTC GTC TTC GTC GGC GCC GAG ATG Ala Ser Ala Gly Met Asn Val Val Phe Val Gly Ala Glu Met 1 5 10	1490
GCG CCG TGG AGC AAG ACC GGC GGC CTC GGC GAC GTC CTC GGC GGC CTG	1538

Ala	Pro	Tr	p Sei	r Lys	5 Th	r Gl	y Gl	y Le	u Gl	y As	p Va	l Le	u Gl	y Gl	y Leu		
15					20					2				-	30		
			C ATO		ì	AAGC	GCGC	GCA	CCGA	GAC .	ATGC.	ATCC	GT T	GGAT	CGCGT	1593	
CTT	CTTC	GTG	CTCT	TGCC	GC C	GTGC	ATGA:	rg cz	ATGT	GTTT(C CT	CCTG	GCTT	GTG'	TTCGT	FT 1653	
ATG:	IGAC	GTG	TTTG	TTCG	GG C	CATGO	CATG	CA G						GTC Val		1705	
			Pro					ı Tyr) Ası		C AGC Ser	1753	
			GAG Glu		CGGC	CAC	CGAG	ACCA	GA I	TCAC	GATCA	C AG	STCAC	CACAC	:	1805	
ACCG	TCA	TAT	GAAC	CTTT	CT C	TGCT	CTGA	T GC	CTGC	AACT	' GCA	AATG	CAT	GCAG	ATC Ile	1862	
AAG Lys															AAG Lys	1910	
CGC Arg																1958	
GTGA	GACG	AG I	ATCT	GATCA	AC TO	CGAT	ACGC	A AT	racc:	ACCC	CAT	rgta.	AGC	AGTT.	ACAGT	G 2018	
AGCT	TTTT	TT (cccc	CCGGC	C TO	GTC	GCTG	G TT	CAG					ACC Thr 100		2072	
GAG <i>F</i> Glu I																2120	
CTG C								GTCA	GGAT	'GG (TTGG	TACI	TA C	AACT:	CATA	2174	

TCATCTGTAT GCAGCAGTAT ACACTGATGA GAAATGCATG CTGTTCTGCA G GCA GCA Ala Ala	2231
CTT GAA GCT CCA AGG ATC CTG AGC CTC AAC AAC AAC CCA TAC TTC TCC Leu Glu Ala Pro Arg Ile Leu Ser Leu Asn Asn Asn Pro Tyr Phe Ser 130 135 140	2279
GGA CCA TAC G GTAAGAGTTG CAGTCTTCGT ATATATATCT GTTGAGCTCG Gly Pro Tyr 145	2329
AGAATCTTCA CAGGAAGCGG CCCATCAGAC GGACTGTCAT TTTACACTGA CTACTGCTGC	2389
TGCTCTTCGT CCATCCATAC AAG GG GAG GAC GTC GTG TTC GTC TGC AAC Gly Glu Asp Val Val Phe Val Cys Asn 150 155	2438
GAC TGG CAC ACC GGC CCT CTC TCG TGC TAC CTC AAG AGC AAC TAC CAG Asp Trp His Thr Gly Pro Leu Ser Cys Tyr Leu Lys Ser Asn Tyr Gln 160 165 170	2486
TCC CAC GGC ATC TAC AGG GAC GCA AAG GTTGCCTTCT CTGAACTGAA	2533
CAACGCCGTT TTCGTTCTCC ATGCTCGTAT ATACCTCGTC TGGTAGTGGT GGTGCTTCTC	2593
TGAGAAACTA ACTGAAACTG ACTGCATGTC TGTCTGACCA TCTTCACGTA CTACCAG	2650
ACC GCT TTC TGC ATC CAC AAC ATC TCC TAC CAG GGC CGG TTC GCC TTC Thr Ala Phe Cys Ile His Asn Ile Ser Tyr Gln Gly Arg Phe Ala Phe 185 190 195	2698
TCC GAC TAC CCG GAG CTG AAC CTC CCG GAG AGA TTC AAG TCG TCC TTC	2746
Ser Asp Tyr Pro Glu Leu Asn Leu Pro Glu Arg Phe Lys Ser Ser Phe 200 205 210	-
GAT TTC ATC GAC GG GTCTGTTTTC CTGCGTGCAT GTGAACATTC ATGAATGGTA Asp Phe Ile Asp Gly 215	2800
ACCCACAACT GTTCGCGTCC TGCTGGTTCA TTATCTGACC TGATTGCATT ATTGCAG C	2858

																	·	
TA	C	GAG	AAG	CCC	GTG	GAA	GGC	CGG	AAG	ATC	AAC	TGG	ATG	AAG	GCC	GGG	-	2906
ту	r	Glu	Lys	Pro	Val	Glu	Gly	Arg	Lys	Ile	Asn	Trp	Met	Lys	Ala	Gly		
			220					225					230					
AT	C	CTC	GAG	GCC	GAC	AGG	GTC	CTC	ACC	GTC	AGC	CCC	TAC	TAC	GCC	GAG	:	2954
Il	e :	Leu	Glu	Ala	Asp	Arg	Val	Leu	Thr	Val	Ser	Pro	Tyr	Tyr	Ala	Glu		
		235					240					245						
GA	G	CTC	ATC	TCC	GGC	ATC	GCC	AGG	GGC	TGC	GAG	CTC	GAC	AAC	ATC	ATG	;	3002
Gl	u :	Leu	Ile	Ser	Gly	Ile	Ala	Arg	Gly	Cys	Glu	Leu	Asp	Asn	Ile	Met		
25	0					255					260					265		
									- .									
CG	C	CTC	ACC	GGC	ATC	ACC	GGC	ATC	GTĆ	AAC	GGC	ATG	GAC	GTC	AGC	GAG	:	3050
Ar	g :	Leu	Thr	Gly	Ile	Thr	Gly	Ile	Val	Asn	Gly	Met	Asp	Val	Ser	Glu		
					270					275					280			
_																		
TG	G	GAC	CCC	AGC	AGG	GAC	AAG	TAC	ATC	GCC	GTG	AAG	TAC	GAC	GTG	TCG		3098
Tr	p.	Asp	Pro	Ser	Arg	Asp	Lys	Tyr	Ile	Ala	Val	Lys	Tyr	Asp	Val	Ser		
				285					290					295				
AC	G	GTG	AGCT	GC :	rage:	CTG	AT TO	CTGC:	rgcc	r GG:	CCT	CCTG	CTC	ATCA:	rgc		;	3151
Th	r																	
TG	GT'	TCG	GTA (CTGA	CGCGG	GC A	AGTG:	racg:	r acc	STGC	GTGC	GAC	GTG	GTG 1	rccg	GTTCA	.G	3211
TG	GT'	TCG	GTA (CTGA	CGCGG	GC A	AGTG	racgi	r ac	GTGC	STGC	GAC	GGTGG	GTG :	rccg	GTTCA	ıG .	3211
					CGCGC													3211 3259
GC	C	GTG	GAG	GCC		GCG	CTG	AAC	AAG	GAG	GCG	CTG	CAG	GCG	GAG	GTC		
GC	C (GTG	GAG	GCC	AAG	GCG	CTG	AAC	AAG	GAG	GCG	CTG	CAG	GCG	GAG	GTC		
GC	C (GTG Val	GAG	GCC	AAG	GCG	CTG Leu	AAC	AAG	GAG	GCG	CTG Leu	CAG	GCG	GAG	GTC		
GC Al	C (GTG Val 300	GAG Glu	GCC Ala	AAG	GCG Ala	CTG Leu 305	AAC Asn	AAG Lys	GAG Glu	GCG Ala	CTG Leu 310	CAG Gln	GCG Ala	GAG Glu	GTC Val	,	
GC Al	C (GTG Val 300 CTC	GAG Glu CCG	GCC Ala	AAG Lys	GCG Ala CGG	CTG Leu 305	AAC Asn	AAG Lys	GAG Glu CTG	GCG Ala	CTG Leu 310 GCG	CAG Gln TTC	GCG Ala	GAG Glu GGC	GTC Val	,	3259
GC Al	C (GTG Val 300 CTC	GAG Glu CCG	GCC Ala	AAG Lys GAC	GCG Ala CGG	CTG Leu 305	AAC Asn	AAG Lys	GAG Glu CTG	GCG Ala	CTG Leu 310 GCG	CAG Gln TTC	GCG Ala	GAG Glu GGC	GTC Val	,	3259
GC Al GG G1	C (GTG Val 300 CTC	GAG Glu CCG	GCC Ala	AAG Lys GAC	GCG Ala CGG Arg	CTG Leu 305	AAC Asn	AAG Lys	GAG Glu CTG	GCG Ala GTG Val	CTG Leu 310 GCG	CAG Gln TTC	GCG Ala	GAG Glu GGC	GTC Val AGG Arg	,	3259
GC Al GG G1 31	G (Y) 5	GTG Val 300 CTC Leu	GAG Glu CCG Pro	GCC Ala GTG Val	AAG Lys GAC	GCG Ala CGG Arg 320	CTG Leu 305 AAC Asn	AAC Asn ATC Ile	AAG Lys CCG Pro	GAG Glu CTG Leu	GCG Ala GTG Val 325	CTG Leu 310 GCG Ala	CAG Gln TTC Phe	GCG Ala ATC Ile	GAG Glu GGC Gly	GTC Val AGG Arg 330		3259
GC Al GG G1 31	C G G G G G G G G G G G G G G G G G G G	GTG Val 300 CTC Leu	GAG Glu CCG Pro	GCC Ala GTG Val	AAG Lys GAC Asp	GCG Ala CGG Arg 320	CTG Leu 305 AAC Asn	AAC Asn ATC Ile	AAG Lys CCG Pro	GAG Glu CTG Leu	GCG Ala GTG Val 325 GCG	CTG Leu 310 GCG Ala	CAG Gln TTC Phe	GCG Ala ATC Ile	GAG Glu GGC Gly	GTC Val AGG Arg 330 CAG		3259 3307
GC Al GG G1 31	C G G G G G G G G G G G G G G G G G G G	GTG Val 300 CTC Leu	GAG Glu CCG Pro	GCC Ala GTG Val	AAG Lys GAC Asp	GCG Ala CGG Arg 320	CTG Leu 305 AAC Asn	AAC Asn ATC Ile	AAG Lys CCG Pro	GAG Glu CTG Leu	GCG Ala GTG Val 325 GCG	CTG Leu 310 GCG Ala	CAG Gln TTC Phe	GCG Ala ATC Ile	GAG Glu GGC Gly	GTC Val AGG Arg 330 CAG		3259 3307
GGC All GGG GI 311 CTT Lee	G Y S	GTG Val 300 CTC Leu GAA Glu	GAG Glu CCG Pro GAG Glu	GCC Ala GTG Val CAG Gln	AAG Lys GAC Asp AAG Lys 335	GCG Ala CGG Arg 320 GGC Gly	CTG Leu 305 AAC Asn CCC Pro	AAC Asn ATC Ile GAC Asp	AAG Lys CCG Pro GTC Val	GAG Glu CTG Leu ATG Met 340	GCG Ala GTG Val 325 GCG Ala	CTG Leu 310 GCG Ala GCC Ala	CAG Gln TTC Phe GCC Ala	GCG Ala ATC Ile ATC	GAG Glu GGC Gly CCG Pro 345	GTC Val AGG Arg 330 CAG Gln		3259 3307 3355
GGC All GGG GI 311 CTT Lee	G Y S	GTG Val 300 CTC Leu GAA Glu	GAG Glu CCG Pro GAG Glu	GCC Ala GTG Val CAG Gln	AAG Lys GAC Asp AAG Lys	GCG Ala CGG Arg 320 GGC Gly	CTG Leu 305 AAC Asn CCC Pro	AAC Asn ATC Ile GAC Asp	AAG Lys CCG Pro GTC Val	GAG Glu CTG Leu ATG Met 340	GCG Ala GTG Val 325 GCG Ala	CTG Leu 310 GCG Ala GCC Ala	CAG Gln TTC Phe GCC Ala	GCG Ala ATC Ile ATC	GAG Glu GGC Gly CCG Pro 345	GTC Val AGG Arg 330 CAG Gln		3259 3307
GGC All GGG GI 311 CTT Lec	G G G G G G G G G G G G G G G G G G G	GTG Val 300 CTC Leu GAA Glu	GAG Glu CCG Pro GAG Glu	GCC Ala GTG Val CAG Gln	AAG Lys GAC Asp AAG Lys 335	GCG Ala CGG Arg 320 GGC Gly	CTG Leu 305 AAC Asn CCC Pro	AAC Asn ATC Ile GAC Asp	AAG Lys CCG Pro GTC Val	GAG Glu CTG Leu ATG Met 340	GCG Ala GTG Val 325 GCG Ala	CTG Leu 310 GCG Ala GCC Ala	CAG Gln TTC Phe GCC Ala	GCG Ala ATC Ile ATC	GAG Glu GGC Gly CCG Pro 345	GTC Val AGG Arg 330 CAG Gln		3259 3307 3355
GGC All GGG G1 31 CT Le	G G G G G G G G G G G G G G G G G G G	GTG Val 300 CTC Leu GAA Glu	GAG Glu CCG Pro GAG Glu	GCC Ala GTG Val CAG Gln	AAG Lys GAC Asp AAG Lys 335	GCG Ala CGG Arg 320 GGC Gly	CTG Leu 305 AAC Asn CCC Pro	AAC Asn ATC Ile GAC Asp	AAG Lys CCG Pro GTC Val	GAG Glu CTG Leu ATG Met 340	GCG Ala GTG Val 325 GCG Ala	CTG Leu 310 GCG Ala GCC Ala	CAG Gln TTC Phe GCC Ala	GCG Ala ATC Ile ATC	GAG Glu GGC Gly CCG Pro 345	GTC Val AGG Arg 330 CAG Gln		3259 3307 3355
GGC All GGG G1 31 CT Le	G G G G G G G G G G G G G G G G G G G	GTG Val 300 CTC Leu GAA Glu	GAG Glu CCG Pro GAG Glu	GCC Ala GTG Val CAG Gln ATG Met	AAG Lys GAC Asp AAG Lys 335	GCG Ala CGG Arg 320 GGC Gly	CTG Leu 305 AAC Asn CCC Pro	AAC Asn ATC Ile GAC Asp	AAG Lys CCG Pro GTC Val	GAG Glu CTG Leu ATG Met 340	GCG Ala GTG Val 325 GCG Ala	CTG Leu 310 GCG Ala GCC Ala	CAG Gln TTC Phe GCC Ala	GCG Ala ATC Ile ATC	GAG Glu GGC Gly CCG Pro 345	GTC Val AGG Arg 330 CAG Gln		3259 3307 3355
GCC All GGG G1 31 CT Lee	G G G U G G U G G U G G U G G U G G G U G G G U G	GTG Val 300 CTC Leu GAA Glu ATG Met	GAG Glu CCG Pro GAG Glu	GCC Ala GTG Val CAG Gln ATG Met 350	AAG Lys GAC Asp AAG Lys 335 GTG Val	GCG Ala CGG Arg 320 GGC Gly	CTG Leu 305 AAC Asn CCC Pro	AAC Asn ATC Ile GAC Asp	AAG Lys CCG Pro GTC Val CAG Gln 355	GAG Glu CTG Leu ATG Met 340 ATC	GCG Ala GTG Val 325 GCG Ala GTT	CTG Leu 310 GCG Ala GCC Ala	CAG Gln TTC Phe GCC Ala CTG Leu	GCG Ala ATC Ile ATC	GAG Glu GGC Gly CCG Pro 345	GTC Val AGG Arg 330 CAG Gln		3259 3307 3355
GCC All GGG G1 31 CT Lee	G G G U G G U G G U G G U G G U G G G U G G G U G	GTG Val 300 CTC Leu GAA Glu ATG Met	GAG Glu CCG Pro GAG Glu	GCC Ala GTG Val CAG Gln ATG Met 350	AAG Lys GAC Asp AAG Lys 335 GTG Val	GCG Ala CGG Arg 320 GGC Gly	CTG Leu 305 AAC Asn CCC Pro	AAC Asn ATC Ile GAC Asp	AAG Lys CCG Pro GTC Val CAG Gln 355	GAG Glu CTG Leu ATG Met 340 ATC	GCG Ala GTG Val 325 GCG Ala GTT	CTG Leu 310 GCG Ala GCC Ala	CAG Gln TTC Phe GCC Ala CTG Leu	GCG Ala ATC Ile ATC	GAG Glu GGC Gly CCG Pro 345	GTC Val AGG Arg 330 CAG Gln		3259 3307 3355

Gly Thr Gly Lys Lys Lys Phe Glu Arg 360 365

ATG CTC ATG AGC GCC GAG GAG AAG TTC CCA GGC AAG GTG CGC GCC GTG	3564
Met Leu Met Ser Ala Glu Glu Lys Phe Pro Gly Lys Val Arg Ala Val	
370 375 380	
GTC AAG TTC AAC GCG GCG CTG GCG CAC CAC ATC ATG GCC GGC GCC GAC	3612
Val Lys Phe Asn Ala Ala Leu Ala His His Ile Met Ala Gly Ala Asp	
385 390 395 400	
GTG CTC GCC GTC ACC AGC CGC TTC GAG CCC TGC GGC CTC ATC CAG CTG	3660
Val Leu Ala Val Thr Ser Arg Phe Glu Pro Cys Gly Leu Ile Gln Leu	
405 410 415	
CAG GGG ATG CGA TAC GGA ACG GTACGAGAGA AAAAAAAAAT CCTGAATCCT	3711
Gln Gly Met Arg Tyr Gly Thr	
420	
GACGAGAGGG ACAGAGACAG ATTATGAATG CTTCATCGAT TTGAATTGAT TGATCGATGT	3771
CTCCCGCTGC GACTCTTGCA G CCC TGC GCC TGC GCG TCC ACC GGT GGA CTC	3822
Pro Cys Ala Cys Ala Ser Thr Gly Gly Leu	
425 430	
GTC GAC ACC ATC ATC GAA GGC AAG ACC GGG TTC CAC ATG GGC CGC CTC	3870
Val Asp Thr Ile Ile Glu Gly Lys Thr Gly Phe His Met Gly Arg Leu	
435 440 445	
AGC GTC GAC GTAAGCCTAG CTCTGCCATG TTCTTTCTTC TTTCTTTCTG	3919
Ser Val Asp	
450	•
TATGTATGTA TGAATCAGCA CCGCCGTTCT TGTTTCGTCG TCGTCCTCTC TTCCCAG	3976
MCM 330 000 000 000	
TGT AAC GTC GTG GAG CCG GCG GAC GTC AAG AAG GTG GCC ACC ACA TTG	4024
Cys Asn Val Val Glu Pro Ala Asp Val Lys Lys Val Ala Thr Thr Leu	
455 460 465	
CAG CGC GCC ATC AAG GTG GTC GGC ACG CCG GCG TAC GAG GAG ATG GTG	4072
Gln Arg Ala Ile Lys Val Val Gly Thr Pro Ala Tyr Glu Glu Met Val	
470 475 480	
NCC NAC MCC AMO AMO AND	
AGG AAC TGC ATG ATC CAG GAT CTC TGC TGG AAG GTACGTACGC CCGCCCCGCC	4125
Arg Asn Cys Met Ile Gln Asp Leu Ser Trp Lys	

CCGCCCCGCC AGAGCAGAGC GCCAAGATCG ACCGATCGAC CGACCACACG TACGCGCCTC	4185
GCTCCTGTCG CTGACCGTGG TTTAATTTGC GAAATGCGCA G GGC CCT GCC AAG Gly Pro Ala Lys	4238
AAC TGG GAG AAC GTG CTG CTC AGC CTC GGG GTC GCC GGC GGC GAG CCA Asn Trp Glu Asn Val Leu Leu Ser Leu Gly Val Ala Gly Gly Glu Pro 500 505 510 515	4286
GGG GTC GAA GGC GAG GAG ATC GCG CCG CTC GCC AAG GAG AAC GTG GCC Gly Val Glu Glu Glu Ile Ala Pro Leu Ala Lys Glu Asn Val Ala 520 525 530	4334
GCG CCC TGA AGAGTTCGGC CTGCAGGGCC CCTGATCTCG CGCGTGGTGC Ala Pro *	4383
AAAGATGTTG GGACATCTTC TTATATATGC TGTTTCGTTT ATGTGATATG GACAAGTATG	4443
TGTAGCTGCT TGCTTGTGCT AGTGTAATGT AGTGTAGTGG TGGCCAGTGG CACAACCTAA	4503
TAAGCGCATG AACTAATTGC TTGCGTGTGT AGTTAAGTAC CGATCGGTAA TTTTATATTG	4563
CGAGTAAATA AATGGACCTG TAGTGGTGGA GTAAATAATC CCTGCTGTTC GGTGTTCTTA	4623
TCGCTCCTCG TATAGATATT ATATAGAGTA CATTTTTCTC TCTCTGAATC CTACGTTTGT	4683
GAAATTTCTA TATCATTACT GTAAAATTTC TGCGTTCCAA AAGAGACCAT AGCCTATCTT	4743
IGGCCCTGTT TGTTTCGGCT TCTGGCAGCT TCTGGCCACC AAAAGCTGCT GCGGACT	4800

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 534 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ala Ser Ala Gly Met Asn Val Val Phe Val Gly Ala Glu Met Ala Pro 1 5 10 15	
Trp Ser Lys Thr Gly Gly Leu Gly Asp Val Leu Gly Gly Leu Pro Pro 20 25 30	
Ala Met Ala Ala Asn Gly His Arg Val Met Val Val Ser Pro Arg Tyr 35 40 45	
Asp Gln Tyr Lys Asp Ala Trp Asp Thr Ser Val Val Ser Glu Ile Lys 50 55 60	
Met Gly Asp Gly Tyr Glu Thr Val Arg Phe Phe His Cys Tyr Lys Arg 65 70 75 80	
Gly Val Asp Arg Val Phe Val Asp His Pro Leu Phe Leu Glu Arg Val	
Trp Gly Lys Thr Glu Glu Lys Ile Tyr Gly Pro Val Ala Gly Thr Asp 100 105 110	
Tyr Arg Asp Asn Gln Leu Arg Phe Ser Leu Leu Cys Gln Ala Ala Leu 115 120 125	
Glu Ala Pro Arg Ile Leu Ser Leu Asn Asn Asn Pro Tyr Phe Ser Gly 130 135 140	
Pro Tyr Gly Glu Asp Val Val Phe Val Cys Asn Asp Trp His Thr Gly 145 150 155 160	
Pro Leu Ser Cys Tyr Leu Lys Ser Asn Tyr Gln Ser His Gly Ile Tyr 165 170 175	
Arg Asp Ala Lys Thr Ala Phe Cys Ile His Asn Ile Ser Tyr Gln Gly 180 185 190	
Arg Phe Ala Phe Ser Asp Tyr Pro Glu Leu Asn Leu Pro Glu Arg Phe 195 200 205	
Lys Ser Ser Phe Asp Phe Ile Asp Gly Tyr Glu Lys Pro Val Glu Gly 210 215 220	
Arg Lys Ile Asn Trp Met Lys Ala Gly Ile Leu Glu Ala Asp Arg Val 225 230 235 240	

Leu Thr Val Ser Pro Tyr Tyr Ala Glu Glu Leu Ile Ser Gly Ile Ala Arg Gly Cys Glu Leu Asp Asn Ile Met Arg Leu Thr Gly Ile Thr Gly Ile Val Asn Gly Met Asp Val Ser Glu Trp Asp Pro Ser Arg Asp Lys Tyr Ile Ala Val Lys Tyr Asp Val Ser Thr Ala Val Glu Ala Lys Ala Leu Asn Lys Glu Ala Leu Gln Ala Glu Val Gly Leu Pro Val Asp Arg Asn Ile Pro Leu Val Ala Phe Ile Gly Arg Leu Glu Glu Gln Lys Gly Pro Asp Val Met Ala Ala Ala Ile Pro Gln Leu Met Glu Met Val Glu Asp Val Gln Ile Val Leu Leu Gly Thr Gly Lys Lys Phe Glu Arg Met Leu Met Ser Ala Glu Glu Lys Phe Pro Gly Lys Val Arg Ala Val Val Lys Phe Asn Ala Ala Leu Ala His His Ile Met Ala Gly Ala Asp Val Leu Ala Val Thr Ser Arg Phe Glu Pro Cys Gly Leu Ile Gln Leu Gln Gly Met Arg Tyr Gly Thr Pro Cys Ala Cys Ala Ser Thr Gly Gly Leu Val Asp Thr Ile Ile Glu Gly Lys Thr Gly Phe His Met Gly Arg Leu Ser Val Asp Cys Asn Val Val Glu Pro Ala Asp Val Lys Lys Val Ala Thr Thr Leu Gln Arg Ala Ile Lys Val Val Gly Thr Pro Ala Tyr

Glu Glu	Met Val	Arg 485	Asn	Cys	Met	Ile	Gln 490	Asp	Leu	Ser	Trp	Lys 495	Gly		
Pro Ala	Lys Asn 500		Glu	Asn	Val	Leu 505	Leu	Ser	Leu	Gly	Val 510	Ala	Gly		
Gly Glu	Pro Gly 515	Val	Glu	Gly	Glu 520	Glu	Ile	Ala	Pro	Leu 525	Ala	Lys	Glu		
Asn Val 1 530	Ala Ala	Pro	*												
(2) INFOR	RMATION	FOR	SEQ	ID N	0:6:	÷									
(i)	SEQUEN	CE CH	ARAC	TERI	STIC	s:									
	(A) L	ENGTH	: 25	42 b	ase	pair	s								
	(B) T														
	(C) S1														
	(D) TO	OPOLO	GY:	not	rele	vant									
(ii)	MOLECUI	LE TY	PE:	CDNA	to i	mRNA									
(iii)	HYPOTHE	TICA	L: No	0											
(vi)	ORIGINA	L SO	JRCE	:											
·	(A) OR				a sat	tiva									
(ix)	FEATURE	:													
	(A) NA	ME/KE	EY: C	CDS				•							
	(B) LO				2282	2									
(xi) :	SEQUENC	E DES	CRIF	PTION	ı: SE	EQ ID	NO:	6:							
GAATTCAGTO	G TGAAG	GAATA	GAT	TCTC	TTC	AAAA	.CAAT	TT A	ATCA	TTCA	T CI	GATO	CTGCT	6	50
CAAAGCTCTC	G TGCAT	CTCCG	GGT	GCAA	.CGG	CCAG	GATA	TT T	ATTG	TGCA	G TA	AAAA	AATG	12	0
TCATATCCC	C TAGCC	ACCCA	AGA	AACT	GCT	CCTT	AAGT	CC T	'TATA	AGCA	C AI	'ATGO	CATT	18	0
GTAATATATA														24	0
AACGTTTTAA														30	0
CCCCAATCCA	AATTGI	CAATA	AAC	TTCA	ATT	CTCC	TAAT'	TA A	CATC	TTAA'	г тс	ATTT	ATTT	36	0

	GA	AAAC	CAGT	TCA	AATTO	CTT 1	TTAC	GCT	CA CO	CAAAC	CTT	A AAC	CAATI	CAA	TTCA	GTGCAG	420
	AG	ATCT:	rcca	CAGO	CAAC	AGC 1	ragac	CAACO	CA CO		: Ser					TCC Ser	473
					Ser					Gly					Ser	GCG Ala	521
				Leu					Phe					Pro		AGC Ser	569
•			Gly					Ser					Thr			CGC Arg	617
		Thr					Arg						AGC Ser				665
													AAC Asn				713
													GGC Gly				761
													GGC Gly 650				809
													GCT Ala				857
													GAG Glu				905
									Val				TTC Phe				953

					AAG Lys				Lys							1001
					GTT Val											1049
					GCA Ala											1097
					AAA Lys 755											1145
					ACT Thr											1193
					ATC Ile											1241
					CAG Gln											1289
					AGG Arg											1337
					GAG Glu 835											1385
					AGG Arg											1433
					ATC Ile											1481
CGG	CTC	ACC	GGC	ATC	ACC	GGC	ATC	GTC	AAC	GGC	ATG	GAC	GTC	AGC	GAG	1529

Arg	Leu	Thr 880	Gly	Ile	Thr	Gly	Ile 885	Val	Asn	Gly	Met	Asp 890	Val	Ser	Glu	
TGG	GAT	CCT	AGC	AAG	GAC	AAG	TAC	ATC	ACC	GCC	AAG	TAC	GAÇ	GCA	ACC	1577
Trp	Asp	Pro	Ser	Lys	Asp	Lys	Tyr	Ile	Thr	Ala	Lys	Tyr	Asp	Ala	Thr	
	895					900					905					
ACG	GCA	ATC	GAG	GCG	AAG	GCG	CTG	AAC	AAG	GAG	GCG	TTG	CAG	GCG	GAG	1625
Thr	Ala	Ile	Glu	Ala	Lys	Ala	Leu	Asn	Lys	Glu	Ala	Leu	Gln	Ala	Glu	
910					915					920					925	
	GGT															1673
Ala	Gly	Leu	Pro		Asp	Arg	Lys	Ile		Leu	Ile	Ala	Phe		Gly	
				930					935					940		
	CTG															1721
Arg	Leu	Glu	Glu	Gln	Lys	Gly	Pro		Val	Met	Ala	Ala		Ile	Pro	
			945					950					955			
	CTC															1769
Glu	Leu	Met	Gln	Glu	Asp	Val	Gln	Ile	Val	Leu	Leu		Thr	Gly	Lys	
		960					965					970				
	AAG															1817
Lys	Lys	Phe	Glu	Lys	Leu	Leu	Lys	Ser	Met	Glu	Glu	Lys	Tyr	Pro	Gly	
	975					980					985					
	GTG															1865
Lys	Val	Arg	Ala	Val	Val	Lys	Phe	Asn	Ala			Ala	His	Leu		
990					995					100					1005	
	GCC															1913
Met	Ala	Gly	Ala	Asp	Val	Leu	Ala	Val			Arg	Phe	Glu			
				101					101					102		
															TGC	1961
Gly	Leu	Ile	Gln	Leu	Gln	Gly	Met	Arg	Tyr	Gly	Thr	Pro			Cys	
			102					103					103			
															GGT	2009
Ala	Ser	Thr	Gly	Gly	Leu	Val	Asp	Thr	Val	Ile	Glu	Gly	Lys	Thr	GJA	
		104	0				104	5				105	0			
mma	C 2 C	7 CC	ccc	CCT	C TO C	NCC.	רייר ר	Cac	ሞርር	מ מ	ርጥር	ርጥር	GAC	רכש	AGC	2057
															Ser	200.
File	nis	wer	GIA	ur.a	red	361	4 CL	uab	-ys	دړي	va I	*41	JIU			

1065 1055 1060 GAC GTG AAG AAG GTG GCG GCC ACC CTG AAG CGC GCC ATC AAG GTC GTC 2105 Asp Val Lys Lys Val Ala Ala Thr Leu Lys Arg Ala Ile Lys Val Val 1070 1075 1080 1085 GGC ACG CCG GCG TAC GAG GAG ATG GTC AGG AAC TGC ATG AAC CAG GAC 2153 Gly Thr Pro Ala Tyr Glu Glu Met Val Arg Asn Cys Met Asn Gln Asp 1090 1095 CTC TCC TGG AAG GGG CCT GCG AAG AAC TGG GAG AAT GTG CTC CTG GGC 2201 Leu Ser Trp Lys Gly Pro Ala Lys Asn Trp Glu Asn Val Leu Leu Gly 1105 1110 CTG GGC GTC GCC GGC AGC GCG CCG GGG ATC GAA GGC GAC GAG ATC GCG 2249 Leu Gly Val Ala Gly Ser Ala Pro Gly Ile Glu Gly Asp Glu Ile Ala

CCG CTC GCC AAG GAG AAC GTG GCT GCT CCT TGA AGAGCCTGAG ATCTACATAT 2302
Pro Leu Ala Lys Glu Asn Val Ala Ala Pro *
1135 1140

GGAGTGATTA ATTAATATAG CAGTATATGG ATGAGAGACG AATGAACCAG TGGTTTGTTT 2362

GTTGTAGTGA ATTTGTAGCT ATAGCCAATT ATATAGGCTA ATAAGTTTGA TGTTGTACTC 2422

TTCTGGGTGT GCTTAAGTAT CTTATCGGAC CCTGAATTTA TGTGTGTGGC TTATTGCCAA 2482

TAATATTAAG TAATAAAGGG TTTATTATAT TATTATATAT GTTATATAT ACTAAAAAAA 2542

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 610 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Ser Ala Leu Thr Thr Ser Gln Leu Ala Thr Ser Ala Thr Gly Phe

1 5 10 15

GTÅ	IIe	Ala	Asp 20		Ser	Ala	Pro	Ser 25		Leu	Leu	Arg	His 30	Gly	Phe
Gln	Gly	Leu 35	Lys	Pro	Arg	Ser	Pro 40	Ala	Gly	Gly	Asp	Ala 45	Thr	Ser	Leu
Ser	Val 50	Thr	Thr	Ser	Ala	Arg 55	Ala	Thr	Pro	Lys	Gln 60	Gln	Arg	Ser	Val
Gln 65	Arg	Gly	Ser	Arg	Arg 70	Phe	Pro	Ser	Val	Val 75	Val	Tyr	Ala	Thr	Gly 80
Ala	Gly	Met	Asn	Val 85	Val	Phe	Val	Gly	Ala 90	Glu	Met	Ala	Pro	Trp 95	Ser
Lys	Thr	Gly	Gly 100	Leu	Gly	Asp	Val	Leu 105	Gly	Gly	Leu	Pro	Pro 110	Ala	Met
Ala	Ala	Asn 115	Gly	His	Arg	Val	Met 120	Val	Ile	Ser	Pro	Arg 125	Tyr	Asp	Gln
Tyr	Lys 130	Asp	Ala	Trp	Asp	Thr 135	Ser	Val	Val	Ala	Glu 140	Ile	Lys	Val	Ala
Asp 145	Arg	Tyr	Glu	Arg	Val 150	Arg	Phe	Phe	His	Cys 155	Tyr	Lys	Arg	Gly	Val 160
Asp	Arg	Val	Phe	Ile 165	Asp	His	Pro	Ser	Phe 170	Leu	Glu	Lys	Val	Trp 175	Gly
Lys	Thr	Gly	Glu 180	Lys	Ile	Tyr	Gly	Pro 185	Asp	Thr	Gly	Val	Asp 190	Tyr	Lys
Asp	Asn	Gln 195	Met	Arg	Phe	Ser	Leu 200	Leu	Cys	Gln	Ala	Ala 205	Leu	Glu	Ala
Pro	Arg 210	Ile	Leu	Asn	Leu	Asn 215	Asn	Asn	Pro	Tyr	Phe 220	Lys	Gly	Thr	Tyr
Gly 225	Glu	Asp	Val	Val	Phe 230	Val	Cys	Asn	Asp	Trp 235	His	Thr	Gly	Pro	Leu 240
Ala	Ser	Tyr	Leu	Lys 245	Asn	Asn	Tyr	Gln	Pro 250	Asn	Gly	Ile	Tyr	Arg 255	Asn

Ala	гλа	Val	A1a 260	Phe	Cys	Ile	His	Asn 265		Ser	Tyr	Gln	Gly 270	Arg	Phe
Ala	Phe	Glu 275	Asp	Tyr	Pro	Glu	Leu 280		Leu	Ser	Glu	Arg 285	Phe	Arg	Ser
Ser	Phe 290	Asp	Phe	Ile	Asp	Gly 295		Asp	Thr	Pro	Val 300	Glu	Gly	Arg	Lys
Ile 305	Asn	Trp	Met	Lys	Ala 310	Gly	Ile	Leu	Glu	Ala 315	Asp	Arg	Val	Leu	Thr 320
Val	Ser	Pro	Tyr	Tyr 325	Ala	Glu	Glu	Leu	11e 330		Gly	Ile	Ala	Arg 335	Gly
Cys	Glu	Leu	Asp 340	Asn	Ile	Met	Arg	Leu 345	Thr	Gly	Ile	Thr	Gly 350	Ile	Val
Asn	Gly	Met 355	Asp	Val	Ser	Glu	Trp 360	Asp	Pro	Ser	Lys	Asp 365	Lys	Tyr	Ile
Thr	Ala 370	ГЛа	Tyr	Asp	Ala	Thr 375	Thr	Ala	Ile	Glu	Ala 380	Lys	Ala	Leu	Asn
Lys 385	Glu	Ala	Leu	Gln	Ala 390	Glu	Ala	Gly	Leu	Pro 395	Val	Asp	Arg	Lys	Ile 400
Pro	Leu	Ile	Ala	Phe 405	Ile	Gly	Arg	Leu	Glu 410	Glu	Gln	Lys	Gly	Pro 415	Asp
Val	Met	Ala	Ala 420	Ala	Ile	Pro	Glu	Leu 425	Met	Gln	Glu	Asp	Val 430	Gln	Ile
Val	Leu	Leu 435	Gly	Thr	Gly	Lys	Lys 440	Lys	Phe	Glu	Lys	Leu 445	Leu	Lys	Ser
Met	Glu 450	Glu	Lys	Tyr	Pro	Gly 455	Lys	Val	Arg	Ala	Val 460	Val	Lys	Phe	Asn
Ala 465	Pro	Leu	Ala	His	Leu 470	Ile	Met	Ala	Gly	Ala 475	Asp	Val	Leu	Ala	Val 480
Pro	Ser	Arg	Phe	Glu 485	Pro	Cys	Gly	Leu	Ile 490	Gln	Leu	Gln	Gly	Met 495	Arg

Tyr Gly Thr Pro Cys Ala Cys Ala Ser Thr Gly Gly Leu Val Asp Thr 500 505 510

Val Ile Glu Gly Lys Thr Gly Phe His Met Gly Arg Leu Ser Val Asp 515 520 525

Cys Lys Val Val Glu Pro Ser Asp Val Lys Lys Val Ala Ala Thr Leu 530 535 540

Lys Arg Ala Ile Lys Val Val Gly Thr Pro Ala Tyr Glu Glu Met Val 545 550 555 560

Arg Asn Cys Met Asn Gln Asp Leu Ser Trp Lys Gly Pro Ala Lys Asn 565 570 575

Trp Glu Asn Val Leu Leu Gly Leu Gly Val Ala Gly Ser Ala Pro Gly 580 585 590

Ile Glu Gly Asp Glu Ile Ala Pro Leu Ala Lys Glu Asn Val Ala Ala 595 600 605

Pro * 610

- (2) INFORMATION FOR SEQ ID NO:8:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2007 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: not relevant
 - (ii) MOLECULE TYPE: cDNA to mRNA
 - (iii) HYPOTHETICAL: NO
 - (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Zea mays
 - (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 1..2007
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GCT	GAG	GCT	GAG	GCC	GGG	GGC	AAG	GAC	GCG	CCG	CCG	GAG	AGG	AGC	GGC	48
Ala	Glu	Ala	Glu	Ala	Gly	Gly	Lys	Asp	Ala	Pro	Pro	Glu	Arg	Ser	Gly	
				615					620					625		
GAC	GCC	GCC	AGG	TTG	ccc	CGC	GCT	CGG	CGC	AAT	GCG	GTC	TCC	AAA	CGG	96
Asp	Ala	Ala	Arg	Leu	Pro	Arg	Ala	Arg	Arg	Asn	Ala	Val	Ser	Lys	Arg	
			630					635					640			
						GTC										144
Arg	Asp		Leu	Gln	Pro	Val		Arg	Tyr	Gly	Ser		Thr	Gly	Asn	
		645					650					655				
											600	663	mmc	ccc	CAC	192
						GCG										192
Thr		Arg	Thr	GIY	Ala		ser	cys	GIN	ASII	670	MIG	Leu	MIG	Asp	•
	660					665					670					
CTT	CAC	አ መረ	כישש	CAC	እ ሞ <i>ር</i>	AAG	ጥርር	ATC	GTC	GCC	GCG	CCG	CCG	ACG	AGC	240
						Lys										
675	GIU	116	Vai	Giu	680	נע	001		,	685					690	
0,5					000						۵					
ATA	GTG	AAG	TTC	CCA	GGG	CGC	GGG	CTA	CAG	GAT	GAT	CCT	TCC	CTC	TGG	288
						Arg										
		•		695	-	_			700					705		
GAC	ATA	GCA	CCG	GAG	ACT	GTC	CTC	CCA	GCC	CCG	AAG	CCA	CTG	CAT	GAA	336
Asp	Ile	Ala	Pro	Glu	Thr	Val	Leu	Pro	Ala	Pro	Lys	Pro	Leu	His	Glu	
			710					715					720			
															GTT	384
Ser	Pro	Ala	Val	Asp	Gly	Asp		Asn	Gly	Ile	Ala		Pro	Thr	Val	
		725					730				•	735				
																432
															GGT	432
Glu			Val	Gln	Glu		Thr	Trp	Asp	Pne	750		Tyr	TTE	Gly	
	740					745					/50					
ጥጥጥ	CAC	CAC	CCT	CAC	CAA	GCG	AAG	САТ	GAT	TCC	AGG	G T T	сст	GCA	GAT	480
															Asp	
755	_	GIU			760		-1-			765					770	
, 55																
GAT	GCT	GGT	TCT	TTT	GAA	CAT	TAT	GGG	ACA	ATG	ATI	CTG	GGC	CTI	TGT	528
															Cys	
_		-		775					780					785		
GGG	GAG	AAT	GTI	ATG	AAC	GTG	ATC	GTG	GTG	GCT	GCI	GAA	TGI	TCT	CCA	576

Gly	Glu	Asn	Val 790	Met	Asn	Val	Ile	Val 795	Val	Ala	Ala	Glu	Cys 800	Ser	Pro	· .
TGG Trp	TGC Cys	AAA Lys 805	ACA Thr	GGT Gly	GGT Gly	CTT Leu	GGA Gly 810	GAT Asp	GTT Val	GTG Val	GGA Gly	GCT Ala 815	TTA Leu	CCC Pro	AAG Lys	624
GCT Ala	TTA Leu 820	GCG Ala	AGA Arg	AGA Arg	GGA Gly	CAT His 825	CGT Arg	GTT Val	ATG Met	GTT Val	GTG Val 830	Val	CCA Pro	AGG Arg	TAT Tyr	672
GGG Gly 835	Asp	TAT Tyr	GTG Val	GAA Glu	GCC Ala 840	TTT Phe	GAT Asp	ATG Met	GGA Gly	ATC Ile 845	CGG Arg	AAA Lys	TAC Tyr	TAC Tyr	AAA Lys 850	720
GCT Ala	GCA Ala	. GGA . Gly	CAG Gln	GAC Asp 855	Leu	GAA Glu	GTG Val	AAC Asn	TAT Tyr 860	Phe	CAT	GCA Ala	TTT Phe	ATT Ile 865	GAT Asp	768
GGA Gly	GTC Val	GAC . Asp	TTT Phe	Val	TTC Phe	ATT	GAT Asp	GCC Ala 875	Ser	TTC Phe	CGG	CAC His	: CGT : Arg :880	Gln	GAT Asp	816
GA(ATA	A TAT TYT 885	Gly	GGA Gly	AGT Ser	AGG Arg	CAG Glr 890	Glu	ATC	: ATG	AAC Lys	G CGC B Arg 895	, Met	ATT	TTG Leu	864
TT:	T TG0	s Lys	GT1	G GCT	r GTT a Val	GAG L Glu	Va!	CCI L Pro	TGC Tr	G CAC	GT: Va: 91	l Pro	A TGC	GG?	GGT Gly	912
GT Va 91	l Cy	C TA	c gg/ r gl	A GAS	r GG/ p Gl/ 920	y Ası	TTO	G GTO	TTO	C ATT	a Al	C ATO	G AAN	r TGG	G CAC p His 930	
					o Va					a Ty					T GGG s Gly 5	
TT Le	'A AT	G CA	G TA n Ty 95	r Th	T CG r Ar	C TC	C GT	C CT l Le 95	u Va	C AT.	A CA e Hi	AA TA .s As	C AT n Il 96	e Gl	C CAC	1056
CA G1	G GG .n Gl	C CG	T GG	T CC	T GT	'A CA 11 Hi	T GA s Gl	A TT u Ph	c cc e Pr	G TA	C Al	rg GA et As	C TT	G CI	G AAC	1104

ACT	AAC	CTT	CAA	CAT	TTC	GAG	CTG	TAC	GAT	CCC	GTC	GGT	GGĆ	GAG	CAC	1152
Thr	Asn	Leu	Gln	His	Phe	Glu	Leu	Tyr	Asp	Pro	Val	Gly	Gly	Glu	His	
	980					985					990					
								٠								
GCC	AAC	ATC	TTT	GCC	GCG	TGT	GTT	CTG	AAG	ATG	GCA	GAC	CGG	GTG	GTG	1200
Ala	Asn	Ile	Phe	Ala	Ala	Cys	Val	Leu	Lys	Met	Ala	Asp	Arg	Val	Val	
995					1000	כ				1005	5				1010	
ACT	GTC	AGC	CGC	GGC	TAC	CTG	TGG	GAG	CTG	AAG	ACA	GTG	GAA	GGC	GGC	1248
Thr	Val	Ser	Arg	_	Tyr	Leu	Trp	Glu		_	Thr	Val	Glu	Gly	Gly	
				1019	5			-	1020)				1025	5	
					ATC											1296
Trp	GTĀ	Leu		_	Ile	Ile	Arg			Asp	Trp	Lys			GIA	
			1030)				1035	•				1040	נ		
እ <i>ጥ</i> ጥ	CCT	C 3 3	000	אשר	CAC	C) C	CAC	C3.C	TCC	220	000	220	C.T.C.	63.6	C.T.C	1244
					GAC											1344
116	ALG	1049	-	TTE	Asp	птэ	1050		115	ASII	PLO	1059		ASP	Val	
		104.	,				1030	•				105.	,			
CAC	CTG	CGG	TCG	GAC	GGC	TAC	ACC	AAC	TAC	TCC	CTC	GAG	ACA	CTC	GAC	1392
His	Leu	Arg	Ser	Asp	Gly	Tyr	Thr	Asn	Tyr	Ser	Leu	Glu	Thr	Leu	Asp	
	1060	ס				1069	5				1070)				
GCT	GGA	AAG	CGG	CAG	TGC	AAG	GCG	GCC	CTG	CAG	CGG	GAC	GTG	GGC	CTG	1440
Ala	Gly	Lys	Arg	Gln	Cys	Lys	Ala	Ala	Leu	Gln	Arg	Asp	Val	Gly	Leu	
1075	5				1080)				1085	5				1090	
					GTG											1488
Glu	Val	Arg	Asp	_	Val	Pro	Leu	Leu	_		Ile	Gly	Arg		_	
				1099	•				1100	,				1109	•	
GGA	CAG	AAG	GGC	GTG	GAC	ልጥር	A T C	ccc	GAC	GCG	ATG	CCG	TGG	ልጥሮ	GCG	1536
• • • • • • • • • • • • • • • • • • • •																2330
Glv	Gln	Lvs	Glv	Val	Aso	Ile	Ile	GIV	ASD					115		
Gly	Gln	Lys	Gly 1110		Asp	Ile	Ile	1115	_	VIG	Mec		1120		n.a	
Gly	Gln	Lys	-		Asp	Ile	Ile		_	AIG	Mec		_		AIG	
			1110)	Asp			1115	5				1120)		1584
GGG	CAG	GAC	1110	CAG		GTG	ATG	1115	GGC	ACC	GGC	CCA	1120 CCT	GAC	CTG	1584
GGG	CAG	GAC	1110 GTG Val	CAG	CTG	GTG	ATG	1115 CTG Leu	GGC	ACC	GGC	CCA	1120 CCT Pro	GAC	CTG	1584
GGG	CAG	GAC Asp	1110 GTG Val	CAG	CTG	GTG	ATG Met	1115 CTG Leu	GGC	ACC	GGC	CCA Pro	1120 CCT Pro	GAC	CTG	1584
GGG Gly	CAG Gln	GAC Asp	GTG Val	CAG Gln	CTG	GTG Val	ATG Met 1130	CTG Leu	GGC Gly	ACC Thr	GGC Gly	CCA Pro 1135	CCT Pro	GAC Asp	CTG Leu	1584 1632
GGG Gly GAA	CAG Gln CGA	GAC Asp 1125 ATG Met	GTG Val	CAG Gln CAG	CTG Leu	GTG Val	ATG Met 1130 GAG Glu	CTG Leu	GGC Gly GAG	ACC Thr	GGC Gly CCC	CCA Pro 1135	CCT Pro	GAC Asp GTG	CTG Leu CGC	

GGG TGG GTG	GGG TTC	TCG GTC CTA	ATG GTG C	AT CGC A	TC ACG CC	GGC 1680
Gly Trp Val	Gly Phe	Ser Val Leu	Met Val H	iis Arg I	le Thr Pro	Gly
1155		1160	1	165		1170
GCC AGC GT	CTG GTG	ATG CCC TCC	CGC TTC G	CC GGC G	GG CTG AA	C CAG 1728
Ala Ser Va	L Leu Val	Met Pro Ser	Arg Phe A	la Gly G	ly Leu As	ı Gln
	1175		1180		118	35
		TAC GGC ACC				
Leu Tyr Ala	a Met Ala	Tyr Gly Thr	Val Pro V	/al Val H	lis Ala Va	l Gly
	1190		1195		1200	
			:			
		GTG GCG CCG				
_	_	Val Ala Pro				a Gly
12	05	121	0	1	.215	
						n ama 1073
		GAC CGC GCC				
	o Thr Phe	Asp Arg Ala	Glu Ala F		eu lle Gl	ı val
.1220		1225		1230		
		a.aaa m.a	001 110 0		MC	G AAG 1920
		GAC ACG TAC				
	s Cys Leu	Asp Thr Tyr		iyr Giu G 1245	stu set it	1250
1235		1240		1243		1230
אכיד כידכ כי	a ece cec	GGC ATG TCG	CAG AAC (מירים אמריים	rgg gac ca	C GCG 1968
		Gly Met Ser				
Ser bed Gr	1255	_	1260		12	
	1233	•				
GCT GAG CT	C TAC GAG	GAC GTC CTT	GTC AAG	rac cag T	rgg	2007
		Asp Val Leu				
	1270		1275	-	•	
	· -					

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 669 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Ala Glu Ala Glu Ala Gly Gly Lys Asp Ala Pro Pro Glu Arg Ser Gly

Asp	Ala	Ala	Arg 20	Leu	Pro	Arg	Ala	Arg 25	Arg	Asn	Ala	Val	Ser 30	Lys	Arg
Arg	Asp	Pro 35	Leu	Gln	Pro	Val	Gly 40	Arg	Tyr	Gly	Ser	Ala 45	Thr	Gly	Asn
Thr	Ala 50	Arg	Thr	Gly	Ala	Ala 55	Ser	CÀa	Gln	Asn	Ala 60	Ala	Leu	Ala	Asp
Val 65	Glu	Ile	Val	Glu	Ile 70	Lys	Ser	Ile	Val	Ala 75	Ala	Pro	Pro	Thr	Ser 80
Ile	Val	Lys	Phe	Pro 85	Gly	Arg	Gly	Leu	Gln 90	Asp	Asp	Pro	Ser	Leu 95	Trp
Asp	Ile	Ala	Pro 100	Glu	Thr	Val	Leu	Pro 105	Ala	Pro	Lys	Pro	Leu 110	His	Glu
Ser	Pro	Ala 115	Val	Asp	Gly	Asp	Ser 120	Asn	Gly	Ile	Ala	Pro 125	Pro	Thr	Val
Glu	Pro 130	Leu	Val	Gln	Glu	Ala 135	Thr	Trp	Asp	Phe	Lys 140	Lys	Tyr	Ile	Gly
Phe 145	Asp	Glu	Pro	Asp	Glu 150	Ala	Lys	Asp	Asp	Ser 155	Arg	Val	Gly	Ala	Asp 160
Asp	Ala	Gly	Ser	Phe 165	Glu	His	Tyr	Gly	Thr 170	Met	Ile	Leu	Gly	Leu 175	Суѕ
Gly	Glu	Asn	Val 180	Met	Asn	Val	Ile	Val 185	Val	Ala	Ala	Glu	Cys 190	Ser	Pro
Trp	Cys	Lys 195	Thr	Gly	Gly	Leu	Gly 200	Asp	Val	Val	Gly	Ala 205	Leu	Pro	Lys
Ala	Leu 210	Ala	Arg	Arg	Gly	His 215	Arg	Val	Met	Val	Val 220	Val	Pro	Arg	Tyr
Gly 225	Asp	Tyr	Val	Glu	Ala 230	Phe	Asp	Met	Gly	Ile 235	Arg	Lys	Tyr	Tyr	Lys 240
Ala	Ala	Gly	Gln	Asp	Leu	Glu	Val	Asn	Tyr	Phe	His	Ala	Phe	Ile	Asp

245 250 255

Gly	Val	Asp	Phe	Val	Phe	Ile	Asp	Ala	Ser	Phe	Arg	His	Arg	Gln	Asp
			260					265					270		

- Asp Ile Tyr Gly Gly Ser Arg Gln Glu Ile Met Lys Arg Met Ile Leu 275 280 285
- Phe Cys Lys Val Ala Val Glu Val Pro Trp His Val Pro Cys Gly Gly 290 295 300
- Val Cys Tyr Gly Asp Gly Asn Leu Val Phe Ile Ala Met Asn Trp His 305 310 315 320
- Thr Ala Leu Leu Pro Val Tyr Leu Lys Ala Tyr Tyr Arg Asp His Gly 325 330 335
- Leu Met Gln Tyr Thr Arg Ser Val Leu Val Ile His Asn Ile Gly His 340 345 350
- Gln Gly Arg Gly Pro Val His Glu Phe Pro Tyr Met Asp Leu Leu Asn 355 360 365
- Thr Asn Leu Gln His Phe Glu Leu Tyr Asp Pro Val Gly Glu His 370 375 380
- Ala Asn Ile Phe Ala Ala Cys Val Leu Lys Met Ala Asp Arg Val Val 385 390 395 400
- Thr Val Ser Arg Gly Tyr Leu Trp Glu Leu Lys Thr Val Glu Gly Gly 405 410 415
- Trp Gly Leu His Asp Ile Ile Arg Ser Asn Asp Trp Lys Ile Asn Gly
 420 425 430
- Ile Arg Glu Arg Ile Asp His Gln Glu Trp Asn Pro Lys Val Asp Val
 435 440 445
- His Leu Arg Ser Asp Gly Tyr Thr Asn Tyr Ser Leu Glu Thr Leu Asp 450 455 460
- Ala Gly Lys Arg Gln Cys Lys Ala Ala Leu Gln Arg Asp Val Gly Leu 465 470 475 480
- Glu Val Arg Asp Asp Val Pro Leu Leu Gly Phe Ile Gly Arg Leu Asp

- Gly Gln Lys Gly Val Asp Ile Ile Gly Asp Ala Met Pro Trp Ile Ala 500 505 510
- Gly Gln Asp Val Gln Leu Val Met Leu Gly Thr Gly Pro Pro Asp Leu 515 520 525
- Glu Arg Met Leu Gln His Leu Glu Arg Glu His Pro Asn Lys Val Arg 530 535 540
- Gly Trp Val Gly Phe Ser Val Leu Met Val His Arg Ile Thr Pro Gly 545 550 560
- Ala Ser Val Leu Val Met Pro Ser Arg Phe Ala Gly Gly Leu Asn Gln 565 570 575
- Leu Tyr Ala Met Ala Tyr Gly Thr Val Pro Val Val His Ala Val Gly 580 585 590
- Gly Leu Arg Asp Thr Val Ala Pro Phe Asp Pro Phe Gly Asp Ala Gly 595 600 605
- Leu Gly Trp Thr Phe Asp Arg Ala Glu Ala Asn Lys Leu Ile Glu Val 610 615 620
- Leu Ser His Cys Leu Asp Thr Tyr Arg Asn Tyr Glu Glu Ser Trp Lys 625 630 635 640
- Ser Leu Gln Ala Arg Gly Met Ser Gln Asn Leu Ser Trp Asp His Ala 645 650 655
- Ala Glu Leu Tyr Glu Asp Val Leu Val Lys Tyr Gln Trp 660 665
- (2) INFORMATION FOR SEQ ID NO:10:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2097 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: not relevant
 - (ii) MOLECULE TYPE: cDNA to mRNA

(:	iii)	HYP	OTHE	TICA	L: N	0								ē		
	(vi)	ORI	GINA	L SO	URCE	:										
		(A) OR	GANI	SM:	Zea	mays						٠.			
			with E													
	(1X)		TURE		EY:	CDS										
		•	•		ON:		097									
	(xi)	SEÇ	UENC	E DE	SCRI	PTIC	n: S	EQ I	D NO	:10:						
ልጥር	ccc	GGG	GC A	ATC	тст	TCC	TCG	TCĞ	TCG	GCT	TTT	CTC	CTC	ccc	GTC	48
Met	Pro	Gly	Ala	Ile	Ser	Ser	Ser	Ser	Ser	Ala	Phe	Leu	Leu	Pro	Val	
670					675					680					685	
								ccc	ccc	እርጥ	CTC	сст	CCT	CCT	CTG	96
GCG	TCC	TCC	TCG	CCG	CGG	Arg	AGG	Ara	GIV	Ser	Val	GGT Gly	Ala	Ala	Leu	
MIG	ser	Ser	Ser	690	nry	9	••••	5	695			•		700		
												CAT				144
Arg	Ser	Tyr	Gly 705	Tyr	Ser	Gly	Ala	Glu 710	Leu	Arg	Leu	His	715	Ala	ALG	
			705					, 10								
CGG	GGC	CCG	CCT	CAG	GAT	GGA	GCG	GCG	TCG	GTA	CGC	GCC	GCA	GCG	GCA	192
Arg	Gly	Pro	Pro	Gln	Asp	Gly		Ala	Ser	Val	Arg		Ala	Ala	Ala	
		720					725					730				
CCG	GCC	GGG	GGC	GAA	AGC	GAG	GAG	GCA	GCG	AAG	AGC	TCC	TCC	TCG	TCC	240
															Ser	
	735					740					745					
G N G	666	666	CCT	ርሞጥ	CAG	GGC	AGC	ACG	GCC	AAG	GCT	GTG	GAT	TCT	GCT	288
Gln	Ala	Glv	Ala	Val	Gln	Gly	Ser	Thr	Ala	Lys	Ala	Val	Asp	Ser	Ala	
750		2			755					760					765	
													- C3-C	700	COM	336
TCA	CCT	CCC	AAT	CCT	TTG	ACA Thr	. TCT Ser	GCT Ala	UUG Pro	LVS	Gln	ser	Gln	Ser	GCT Ala	
ser	FEO	FLO	U D		ب ب					-1-						

GCA ATG CAA AAC GGA ACG AGT GGG GGC AGC AGC GCG AGC ACC GCC GCG 384

Ala Met Gln Asn Gly Thr Ser Gly Gly Ser Ser Ala Ser Thr Ala Ala

CCG GTG TCC GGA CCC AAA GCT GAT CAT CCA TCA GCT CCT GTC ACC AAG

Pro	Val	Ser 800	Gly	Pro	Lys	Ala	Asp 805	His	Pro	Ser	Ala	Pro 810	Val	Thr	Lys	· ·
						GCG Ala 820										480
						ATA Ile										528
						ACA Thr		-								576
			-			GGC Gly										624
						GAA Glu										672
						GCT Ala 900										720
						ATA Ile										768
						AGA Arg										816
						TCT Ser										864
						CGG Arg										912
						AAG Lys										960

GTT GAG GTT Val Glu Val		Tyr Ala		Gly Gly	Thr Val		y Asp	1008
990		995		1000)		1005	
GGC AAC TTA								1056
Gly Asn Leu			Asn Asp	-	Thr Ala			
	101	U		1015		10	20	
GTC TAT CTA	AAG GCC	TAT TAC	CGG GAC	AAT GGT	TTG ATG	CAG TA	T GCT	1104
Val Tyr Leu		Tyr Tyr	Arg Asp	Asn Gly	Leu Met	Gln Ty	r Ala	
	1025		103	0		1035		
CGC TCT GTG	CTT GTG	ATA CAC	AAC ATT	GCT CAT	CAG GGT	CGT GG	C CCT	1152
Arg Ser Val	Leu Val	Ile His	Asn Ile	Ala His	Gln Gly	Arg Gl	y Pro	
104	0		1045		1050)		
GTA GAC GAC	TTC GTC	AAT TTT	GAC TTG	CCT GAA	CAC TAC	ATC GA	C CAC	1200
Val Asp Asp	Phe Val	Asn Phe	Asp Leu	Pro Glu	His Tyr	Ile As	p His	
1055		106	0		1065			
TTC AAA CTG	TAT GAC	AAC ATT	GGT GGG	GAT CAC	AGC AAC	GTT TT	T GCT	1248
Phe Lys Leu	Tyr Asp	Asn Ile	Gly Gly	Asp His	Ser Asn	Val Ph	e Ala	
1070		1075,		1080	ס		1085	
GCG GGG CTG	AAG ACG	GCA GAC	CGG GTG	GTG ACC	GTT AGC	AAT GG	C TAC	1296
GCG GGG CTG								1296
		Ala Asp				Asn Gl		1296
	Lys Thr	Ala Asp O	Arg Val	Val Thr 1095	Val Ser	Asn Gl	у Туг 00	1296
Ala Gly Leu	Lys Thr 109 CTG AAG Leu Lys	Ala Asp O ACT TCG	Arg Val	Val Thr 1095 GGG TGG	Val Ser	Asn Gl 11 CAC GA	y Tyr 00 C ATC	
Ala Gly Leu	Lys Thr 109 CTG AAG	Ala Asp O ACT TCG	Arg Val	Val Thr 1095 GGG TGG Gly Trp	Val Ser	Asn Gl 11 CAC GA	y Tyr 00 C ATC	
Ala Gly Leu	Lys Thr 109 CTG AAG Leu Lys 1105	Ala Asp O ACT TCG Thr Ser	Arg Val	Val Thr 1095 GGG TGG Gly Trp	Val Ser GGC CTC Gly Leu	Asn Gl 11 CAC GA His As 1115	y Tyr 00 C ATC p Ile	
Ala Gly Leu ATG TGG GAG Met Trp Glu	Lys Thr 109 CTG AAG Leu Lys 1105 AAC GAC	Ala Asp O ACT TCG Thr Ser	Arg Val	Val Thr 1095 GGG TGG Gly Trp O	Val Ser GGC CTC Gly Leu GTG AAC	Asn Gl 11 CAC GA His As 1115 GGC AT	y Tyr 00 C ATC p Ile C GAC	1344
ATG TGG GAG Met Trp Glu	Lys Thr 109 CTG AAG Leu Lys 1105 AAC GAC Asn Asp	Ala Asp O ACT TCG Thr Ser	Arg Val	Val Thr 1095 GGG TGG Gly Trp O	Val Ser GGC CTC Gly Leu GTG AAC	Asn Gl 11 CAC GA His As 1115 GGC AT Gly II	y Tyr 00 C ATC p Ile C GAC	1344
ATG TGG GAG Met Trp Glu ATA AAC CAG Ile Asn Gln	Lys Thr 109 CTG AAG Leu Lys 1105 AAC GAC Asn Asp	Ala Asp O ACT TCG Thr Ser TGG AAG Trp Lys	GAA GGC Glu Gly 111 CTG CAG Leu Gln 1125	Val Thr 1095 GGG TGG Gly Trp O GGC ATC Gly Ile	Val Ser GGC CTC Gly Leu GTG AAC Val Asn 1136	Asn Gl 11 CAC GA His As 1115 GGC AT Gly Il	y Tyr 00 C ATC p Ile C GAC e Asp	1344
ATG TGG GAG Met Trp Glu ATA AAC CAG Ile Asn Gln	Lys Thr 109 CTG AAG Leu Lys 1105 AAC GAC Asn Asp	Ala Asp O ACT TCG Thr Ser TGG AAG Trp Lys CCC GCT	GAA GGC Glu Gly 111 CTG CAG Leu Gln 1125 GTG GAC	Val Thr 1095 GGG TGG Gly Trp GGC ATC Gly Ile GTG CAC	Val Ser GGC CTC Gly Leu GTG AAC Val Asn 1136 CTC CAC	Asn Gl 11 CAC GA His As 1115 GGC AT Gly I1	y Tyr 00 C ATC p Ile C GAC e Asp	1344
ATA AAC CAG Ile Asn Gln 112	Lys Thr 109 CTG AAG Leu Lys 1105 AAC GAC Asn Asp	Ala Asp O ACT TCG Thr Ser TGG AAG Trp Lys CCC GCT	GAA GGC Glu Gly 111. CTG CAG Leu Gln 1125 GTG GAC Val Asp	Val Thr 1095 GGG TGG Gly Trp GGC ATC Gly Ile GTG CAC	Val Ser GGC CTC Gly Leu GTG AAC Val Asn 1136 CTC CAC	Asn Gl 11 CAC GA His As 1115 GGC AT Gly I1	y Tyr 00 C ATC p Ile C GAC e Asp	1344
ATG TGG GAG Met Trp Glu ATA AAC CAG Ile Asn Gln 112 ATG AGC GAG Met Ser Glu	Lys Thr 109 CTG AAG Leu Lys 1105 AAC GAC Asn Asp 0 TGG AAC	Ala Asp O ACT TCG Thr Ser TGG AAG Trp Lys CCC GCT Pro Ala 114	GAA GGC Glu Gly 111 CTG CAG Leu Gln 1125 GTG GAC Val Asp	Val Thr 1095 GGG TGG Gly Trp GGC ATC Gly Ile GTG CAC Val His	GGC CTC Gly Leu GTG AAC Val Asn 1136 CTC CAC Leu His 1145	Asn Gl 11 CAC GA His As 1115 GGC AT Gly I1 O	y Tyr 00 C ATC p Ile C GAC e Asp C GAC	1344
ATG TGG GAG Met Trp Glu ATA AAC CAG Ile Asn Gln 112 ATG AGC GAG Met Ser Glu 1135	Lys Thr 109 CTG AAG Leu Lys 1105 AAC GAC Asn Asp 0 TGG AAC Trp Asn	Ala Asp O ACT TCG Thr Ser TGG AAG Trp Lys CCC GCT Pro Ala 114 TTC GAG	Arg Val GAA GGC Glu Gly 111 CTG CAG Leu Gln 1125 GTG GAC Val Asp 0 ACG CTG	Val Thr 1095 GGG TGG Gly Trp GGC ATC Gly Ile GTG CAC Val His GAC ACC	Val Ser GGC CTC Gly Leu GTG AAC Val Asn 1136 CTC CAC Leu His 1145	Asn Gl 11 CAC GA His As 1115 GGC AT Gly II O TCC GA Ser As	y Tyr 00 C ATC p Ile C GAC e Asp C GAC p Asp	1344 1392 1440
ATG TGG GAG Met Trp Glu ATA AAC CAG Ile Asn Gln 112 ATG AGC GAG Met Ser Glu 1135	Lys Thr 109 CTG AAG Leu Lys 1105 AAC GAC Asn Asp 0 TGG AAC Trp Asn	Ala Asp O ACT TCG Thr Ser TGG AAG Trp Lys CCC GCT Pro Ala 114 TTC GAG	Arg Val GAA GGC Glu Gly 111 CTG CAG Leu Gln 1125 GTG GAC Val Asp 0 ACG CTG	Val Thr 1095 GGG TGG Gly Trp GGC ATC Gly Ile GTG CAC Val His GAC ACC	Val Ser GGC CTC Gly Leu GTG AAC Val Asn 1136 CTC CAC Leu His 1145 GGC AAG Gly Lys	Asn Gl 11 CAC GA His As 1115 GGC AT Gly II O TCC GA Ser As	y Tyr 00 C ATC p Ile C GAC e Asp C GAC p Asp	1344 1392 1440

							1									•
AAG	GCC	GCC	CTG	CAG	CGG	CAG	CTG	محث	CTG	CAG	GTC	CGC	GAC	GAC	GTG	1536
Lys	Ala	Ala	Leu	Gln	Arg	Gln	Leu	Gly	Le.	Gln	Val	Arg	Asp	Asp	Val	•
				1170)				1179	,				1180)	
CCA	CTG	ATC	GGG	TTC	ATC	GGG	CGG	CTG	GAC	CAC	CAG	AAG	GaC	GTG	GAC	1584
Pro	Leu	Ile	Gly	Phe	Ile	Gly	Arg	Leu	Asp	His	Gln	Lys	Gly	Val	Asp	
			1189	5				1190)				119	5		
ATC	ATC	GCC	GAC	GCG	ATC	CAC	TGG	ATC	GCG	GGG	CAG	GAC	GTG	CAG	CTC	1632
Ile	Ile	Ala	Asp	Ala	Ile	His	Trp	Ile	Ala	Gly	Gln	Asp	Val	Gln	Leu	
		1200					1209					1210				
GTG	ATG	CTG	GGC	ACC	GGG	CGG	GCC	GAC	CTG	GAG	GAC	ATG	CTG	CGG	CGG	1680
						Arg										
	1215		-		_	1220		-			122					
TTC	GAG	TCG	GAG	CAC	AGC	GAC	AAG	GTG	CGC	GCG	TGG	GTG	GGG	TTC	TCG	1728
Phe	Glu	Ser	Glu	His	Ser	Asp	Lys	Val	Arg	Ala	Trp	Val	Gly	Phe	Ser	
1230					1239		-		_	124			_		1245	
GTG	ccc	CTG	GCG	CAC	CGC	ATC	ACG	GCG	GGC	GCG	GAC	ATC	CTG	CTG	ATG	1776
						Ile										
				1250					125		•			126		
				123												
CCG	TCG	CGG	TTC	GAG	CCG	TGC	GGG	CTG	AAC	CAG	CTC	TAC	GCC	ATG	GCG	1824
															Ala	
	202	9	126			-1-	2	127				-1-	127			
TAC	GGG	ACC	GTG	ccc	GTG	GTG	CAC	GCC	GTG	GGG	GGG	CTC	CGG	GAC	ACG	1872
															Thr	
-1-	1	1280					128			•	•	129		•		
GTG	GCG	CCG	TTC	GAC	CCG	TTC	AAC	GAC	ACC	GGG	CTC	GGG	TGG	ACG	TTC	1920
															Phe	
	1299		•			1300		•		•	130		•			
		-										_				
GAC	CGC	GCG	GAG	GCG	AAC	CGG	ATG	ATC	GAC	GCG	CTC	TCG	CAC	TGC	CTC	1968
															Leu	
1310	_		014		131					132				-1-	1325	
	•				±,	-					-					
ACC	ACG	TAC	CGG	AAC	TAC	AAG	GAG	AGC	TGG	CGC	GCC	TGC	AGG	GCG	CGC	2016
															Arg	
		- 1 -	9	133		-13			133			-10	9	134		
				133	-				233	-					-	
GGG	אייים	ccc	CAC	CAC	ርጥር	NG C	TGC	GAC	ראכ	פככ	GCC	ርጥር	ርጥር	ጥልጥ	GAG	2064
700	WIG.		UNU	GAC	- L C	AGC.	7.00	346	CAC	900	300	G 1 G	- 10	T-12-T	~~	2001

Gly	Met	Ala	Glu	Asp	Leu	Ser	Trp	Asp	His	Ala	Ala	Val	Leu	Tyr	Glu
			1345	5				1350)				1355	5	

GAC GTG CTC GTC AAG GCG AAG TAC CAG TGG TGA
Asp Val Leu Val Lys Ala Lys Tyr Gln Trp *
1360 1365

2097

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 699 amino acids
 - (B) TYPE: amino acid :
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Pro Gly Ala Ile Ser Ser Ser Ser Ser Ala Phe Leu Leu Pro Val

Ala Ser Ser Ser Pro Arg Arg Arg Gly Ser Val Gly Ala Ala Leu 20 25 30

Arg Ser Tyr Gly Tyr Ser Gly Ala Glu Leu Arg Leu His Trp Ala Arg

Arg Gly Pro Pro Gln Asp Gly Ala Ala Ser Val Arg Ala Ala Ala Ala 50 55 60

Pro Ala Gly Gly Glu Ser Glu Glu Ala Ala Lys Ser Ser Ser Ser Ser 65 70 75 80

Gln Ala Gly Ala Val Gln Gly Ser Thr Ala Lys Ala Val Asp Ser Ala 85 90 95

Ser Pro Pro Asn Pro Leu Thr Ser Ala Pro Lys Gln Ser Gln Ser Ala 100 105 110

Ala Met Gln Asn Gly Thr Ser Gly Gly Ser Ser Ala Ser Thr Ala Ala 115 120 125

Pro Val Ser Gly Pro Lys Ala Asp His Pro Ser Ala Pro Val Thr Lys 130 135 140

145	GIU	iie	Asp	Ala	150	Ата	vai	гÀа	Pro	155		Ala	GIĀ	Asp	160
Ala	Arg	Pro	Val	Glu 165	Ser	Ile	Gly	Ile	Ala 170		Pro	Val	Asp	Ala 175	Lys
Ala	Asp	Ala	Ala 180	Pro	Ala	Thr	Asp	Ala 185	Ala	Ala	Ser	Ala	Pro 190	Tyr	Asp
Arg	Glu	Asp 195	Asn	Glu	Pro	Gly	Pro 200	Leu	Ala	Gly	Pro	Asn 205	Val	Met	Asn
Val	Val 210	Val	Val	Ala	Ser	Glu 215	Суз	Ala	Pro	Phe	Cys 220	Lys	Thr	Gly	Gly
Leu 225	Gly	Asp	Val	Val	Gly 230	Ala	Leu	Pro	Lys	Ala 235	Leu	Ala	Arg	Arg	Gly 240
His	Arg	Val	Met	Val 245	Val	Ile	Pro	Arg	Tyr 250	Gly	Glu	Tyr	Ala	Glu 255	Ala
Arg	Asp	Leu	Gly 260	Val	Arg	Arg	Arg	Tyr 265	Lys	Val	Ala	Gly	Gln 270	Asp	Ser
Glu [*]	Val	Thr 275	Tyr	Phe	His	Ser	Tyr 280	Ile	Asp	Gly	Val	Asp 285	Phe	Val	Phe
Val	Glu 290	Ala	Pro	Pro	Phe	Arg 295	His	Arg	His	Asn	Asn 300	Ile	Tyr	Gly	Gly
Glu 305	Arg	Leu	Asp	Ile	Leu 310	Lys	Arg	Met	Ile	Leu 315	Phe	Cys	Lys	Ala	Ala 320
Val	Glu	Val	Pro	Trp 325	Tyr	Ala	Pro	Суѕ	Gly 330	Gly	Thr	Val	Tyr	Gly 335	Asp
Gly	Asn	Leu	Val 340	Phe	Ile	Ala	Asn	Asp 345	Trp	His	Thr	Ala	Leu 350	Leu	Pro
Val	Tyr	Leu 355	Lys	Ala	Tyr	Tyr	Arg 360	Asp	Asn	Gly	Leu	Met 365	Gln	Tyr	Ala
Arg	Ser	Val	Leu	Val		His	Asn	Ile	Ala	His	Gln	Gly	Arg	Gly	Pro

385	Asp	Asp	Phe	Val	390	Pne	Asp	Leu	Pro	395	nis	ıyr	iie	Asp	400
Phe	Lys	Leu	Tyr	Asp 405	Asn	Ile	Gly	Gly	Asp 410	His	Ser	Asn	Val	Phe 415	Ala
Ala	Gly	Leu	Lys 420	Thr	Ala	Asp	Arg	Val 425	Val	Thr	Val	Ser	Asn 430	Gly	Tyr
Met	Trp	Glu 435	Leu	Lys	Thr	Ser	Glu 440	Gly	Gly	Trp	Gly	Leu 445	His	Asp	Ile
Ile	Asn 450	Gln	Asn	Asp	Trp	Lys 455	Leu	Gln	Gly	Ile	Val 460	Asn	Gly	Ile	Asp
Met 465	Ser	Glu	Trp	Asn	Pro 470	Ala	Val	Asp	Val	His 475	Leu	His	Ser	Asp	Asp 480
Tyr	Thr	Asn	Tyr	Thr 485	Phe	Glu	Thr	Leu	Asp 490	Thr	Gly	Lys	Arg	Gln 495	Cys
Lys	Ala	Ala	Leu 500	Gln	Arg	Gln	Leu	Gly 505	Leu	Gln	Val	Arg	Asp 510	Asp	Val
Pro	Leu	Ile 515	Gly	Phe	Ile	Gly	Arg 520	Leu	Asp	His	Gln	Lys 525	Gly	Val	Asp
Ile	Ile 530	Ala	Asp	Ala	Ile	His 535	Trp	Ile	Ala	Gly	Gln 540	Asp	Val	Gln	Leu
Val 545	Met	Leu	Gly	Thr	Gly 550	Arg	Ala	Asp	Leu	Glu 555	Asp	Met	Leu	Arg	Arg 560
Phe	Glu	Ser	Glu	His 565	Ser	Asp	Lys	Val	Arg 570	Ala	Trp	Val	Gly	Phe 575	Ser
Val	Pro	Leu	Ala 580	His	Arg	Ile	Thr	Ala 585	Gly	Ala	Asp	Ile	Leu 590	Leu	Met
Pro	Ser	Arg 595	Phe	Glu	Pro	Cys	Gly 600	Leu	Asn	Gln	Leu	Tyr 605	Ala	Met	Ala
Tyr	Gly 610	Thr	Val	Pro	Val	Val 615	His	Ala	Val	Gly	Gly 620	Leu	Arg	Asp	Thr

Val	Ala	Pro	Phe	Asp	Pro	Phe	Asn	Asp	Thr	Gly	Leu	Gly	Trp	Thr	Phe	٠.
625					630					635					640	
Asp	Arg	Ala	Glu	Ala 645	Asn	Arg	Met	Ile	Asp 650	Ala	Leu	Ser	His	Суз 655	Leu	
Thr	Thr	Tyr	Arg 660	Asn	Tyr	ГÀа	Glu	Ser 665	Trp	Arg	Ala	Суз	Arg 670	Ala	Arg	
Gly	Met	Ala 675	Glu	Asp	Leu	Ser	Trp 680	Asp	His	Ala	Ala	Val 685	Leu	Tyr	Glu	
Asp	Val 690	Leu	Val	Lys	Ala	695	Tyr	Gln	Trp	*					. ·	
(2)	INFO	ORMA	rion	FOR	SEQ	ID 1	10:12	2:								
	(ii) (iii) (vi)	() (I) (I) (I) (I) (I) (I) (I) (I) (I) (A) LH B) T: C) S: C) T:	ENGTH (PE: IRANI DPOLO LE TY ETICA AL SO RGAN: E: AME/I	HARACHE 17 nuclosed N	752 k Leic ESS: not CDN? NO	acio douk rele A to may:	pair i ole evant mRNA	=							
	(xi) SEÇ	QUENC	CE DI	ESCRI	PTIC	ON: 5	SEQ 1	ID NO	0:12:	:					
					AGC Ser 705											48
					CCC Pro											96

GCC	GAG	CCC	ACG	GGT	GAG	CCG	GCA	TCG	ACG	CCG	CCG	CCC	GTG	CCC	GAC		144
Ala	Glu	Pro	Thr	Gly	Glu	Pro	Ala	Ser	Thr	Pro	Pro	Pro	Val	Pro	Asp		
			735					740					745				
GCC	GGC	CTG	GGG	GAC	CTC	GGT	CTC	GAA	CCT	GAA	GGG	ATT	GCT	GAA	GGT		192
Ala	Gly	Leu	Gly	Asp	Leu	Gly	Leu	Glu	Pro	Glu	Gly	Ile	Ala	Glu	Gly		
		750					755					760					
TCC	ATC	GAT	AAC	ACA	GTA	GTT	GTG	GCA	AGT	GAG	CAA	GAT	TCT	GAG	ATT		240
Ser	Ile	Asp	Asn	Thr	Val	Val	Val	Ala	Ser	Glu	Gln	Asp	Ser	Glu	Ile		
	765					770					775						
GTG	GTT	GGA	AAG	GAG	CAA	GCT	CGA	GCT.	AAA	GTA	ACA	CAA	AGC	ATT	GTC		288
Val	Val	Gly	Lys	Glu	Gln	Ala	Arg	Ala	Lys	Val	Thr	Gln	Ser	Ile	Val		
780					785					790					795	•	
TTT	GTA	ACC	GGC	GAA	GCT	TCT	CCT	TAT	GCA	AAG	TCT	GGG	GGT	CTA	GGA		336
Phe	Val	Thr	Gly	Glu	Ala	Ser	Pro	Tyr	Ala	Lys	Ser	Gly	Gly	Leu	Gly		
				800					805					810			
GAT	GTT	TGT	GGT	TCA	TTG	CCA	GTT	GCT	CTT	GCT	GCT	CGT	GGT	CAC	CGT		384
Asp	Val	Cys	Gly	Ser	Leu	Pro	Val	Ala	Leu	Ala	Ala	Arg	Gly	His	Arg		
			815					820					825				
GTG	ATG	GTT	GTA	ATG	CCC	AGA	TAT	TTA	AAT	GGT	ACC	TCC	GAT	AAG	AAT		432
Val	Met	Val	Val	Met	Pro	Arg	Tyr	Leu	Asn	Gly	Thr	Ser	Asp	Lys	Asn		
		830					835					840					
			GCA														480
Tyr		Asn	Ala	Phe	Tyr	Thr	Glu	Lys	His	Ile	Arg	Ile	Pro	Cys	Phe		
	845					850					855						
			CAT									•					528
	GLY	Glu	His	Glu		Thr	Phe	Phe	His		Tyr	Arg	Asp	Ser			*
860					865					870					875		
			TTT														576
Asp	Trp	vai	Phe		Asp	His	Pro	ser	_	HIS	Arg	Pro	GIY		Leu		
				880					885					890			
m n m	CCN	a						~~~	a.m								c 2 4
			AAG														624
- A -	GTÅ	Asp	Lys 895	rne	GTÀ	WIG	rne		ASD	ASN	GTU	rne	_	Tyr	rnr		
			073					900					905				
CTC	ርጥጥ	ፐርር	TAT	COT	GC N	ጥርጥ	GAC	CCT	CCT	ጥጥረ	አ ጥ ~	COMO	C 3 3	ጥጥረ	CCS		672
CIC	C11	160	IAI	GCI	GCA	IGI	GAG	GCT	CCT	116	ATC	CTT	GAA	TTG	GGA		0/2

Leu	Leu	Cys 910	Tyr	Ala	Ala	Cys	Glu 915	Ala	Pro	Leu	Ile	Leu 920	Glu	Leu	Gly	÷.
GGA	TAT	ATT	TAT	GGA	CAG	AAT	TGC	ATG	TTT	GTT	GTC	AAT	GAT	TGG	CAT	720
Gly	Tyr	Ile	Tyr	Gly	Gln	Asn	Cys	Met	Phe	Val	Val	Asn	Asp	Trp	His	
	925					930					935					
GCC	AGT	CTA	GTG	CCA	GTC	CTT	CTT	GCT	GCA	AAA	TAT	AGA	CCA	TAT	GGT	768
Ala	Ser	Leu	Val	Pro	Val	Leu	Leu	Ala	Ala	Lys	Tyr	Arg	Pro	Tyr	Gly	
940					945					950					955	
GTT	TAT	AAA	GAC	TCC	CGC	AGC	ATT	CTT	GTA	ATA	CAT	AAT	TTA	GCA	CAT	816
Val	Tyr	Lys	Asp	Ser	Arg	Ser	Ile	Leu	Val	Ile	His	Asn	Leu	Ala	His	
				960					965					970		
CAG	GGT	GTA	GAG	CCT	GCA	AGC	ACA	TAT	CCT	GAC	CTT	GGG	TTG	CCA	CCT	864
Gln	Gly	Val	Glu	Pro	Ala	Ser	Thr	Tyr	Pro	Asp	Leu	Gly	Leu	Pro	Pro	
			975					980					985			
						GAG										912
Glu	Trp		Gly	Ala	Leu	Glu	Trp	Val	Phe	Pro	Glu	Trp	Ala	Arg	Arg	
		990					995					1000)			
CAT	GCC	CTT	GAC	AAG	GGT	GAG	GCA	GTT	AAT	TTT	TTG	AAA	GGT	GCA	GTT	960
His			Asp	Lys	Gly	Glu	Ala	Val	Asn	Phe	Leu	Lys	Gly	Ala	Val	
	1009	5				1010)				1015	5				
						GTG										1008
		Ala	Asp	Arg	Ile	Val	Thr	Val	Ser	Lys	Gly	Tyr	Ser	Trp	Glu	
1020)				1025	5				1030)				1035	
						GGA										1056
Val	Thr	Thr	Ala			Gly	Gln	Gly	Leu	Asn	Glu	Leu	Leu	Ser	Ser	
				1040)				1045	•				1050	ס	
AGA.	AAG	AGT	GTA	TTA	AAC	GGA	ATT	GTA	AAT	GGA	ATT	GAC	ATT	AAT	GAT	1104
Arg	Lys	Ser	Val	Leu	Asn	Gly	Ile	Val	Asn	Gly	Ile	Asp	Ile	Asn	Asp	
			1055	i				1060)				1065	5		
TGG	AAC	CCT	GCC	ACA	GAC	AAA	TGT	ATC	CCC	TGT	CAT	TAT	TCT	GTT	GAT	1152
Trp	Asn	Pro	Ala	Thr	Asp	Lys	Cys	Ile	Pro	Cys	His	Tyr	Ser	Val	Asp	
		1070)				1075	5				1080)			
GAC	CTC	TCT	GGA	AAG	GCC	AAA	TGT	AAA	GGT	GCA	TTG	CAG	AAG	GAG	CTG	1200
														Glu		

																•
GGI	' TTA	CCI	ATA	AGG	CCT	GAT	GTT	CCT	CTG	ATT	GGC	TTT	ATT	GGA	AGG	1248
Gly	Leu	Pro	Ile	Arg	Pro	Asp	Val	Pro	Leu	Ile	Gly	Phe	Ile	Gly	Arg	
110	0				110	5				111	.0				1115	
TTG	GAT	TAT	CAG	AAA	GGC	ATT	GAT	CTC	ATT	CAA	CTT	ATC	ATA	CCA	GAT	1296
Leu	Asp	Tyr	Gln	Lys	Gly	Ile	Asp	Leu	Ile	Gln	Leu	Ile	Ile	Pro	Asp	
				112	0				112	5				113	0	
CTC	ATG	CGG	GAA	GAT	GTT	CAA	TTT	GTC	ATG	CTT	GGA	TCT	GGT	GAC	CCA	1344
Leu	Met	Arg	Glu	Asp	Val	Gln	Phe	Val	Met	Leu	Gly	Ser	Gly	Asp	Pro	
			113	5				114	Ö				114	5		
GAG	CTT	GAA	GAT	TGG	ATG	AGA	TCT	ACA	GAG	TCG	ATC	TTC	AAG	GAT	AAA	1392
			Asp													
		115					115					116	_	•	-1	
TTT	CGT	GGA	TGG	GTT	GGA	TTT	AGT	GTT	CCA	GTT	TCC	CAC	CGA	ATA	ACT	1440
			Trp													
	116		_		•	1170					117		9			
GCC	GGC	TGC	GAT	ATA	TTG	TTA	ATG	CCA	TCC	AGA	TTC	GAA	ССТ	тст	сст	1488
			Asp													1400
118		-	•		1185					1190				O _f S	1195	
											_				1173	
CTC	AAT	CAG	CTA	TAT	GCT	ATG	CAG	TAT	GGC	ACA	стт	ССТ	GTT	GTC	СУТ	1536
			Leu													1330
				1200				•	1209					1210		
														1210	•	
GCA	ACT	GGG	GGC	CTT	AGA	GAT	ACC	GTG	GAG	AAC	TTC	AAC	ССТ	ጥጥር	GGT	1584
			Gly													1304
		-	1215			•		1220					1225		GLY	
														•		
GAG	AAT	GGA	GAG	CAG	GGT	ACA	GGG	TGG	GCA	TTC	GCA	CCC	СТА	ACC	ACA	1632
			Glu													1032
		1230			•		1235					1240				
GAA	AAC	ATG	TTT	GTG	GAC	ATT	GCG	AAC	TGC	ААТ	ATC	TAC	АТА	CAG	GGA	1680
			Phe													1000
	1245				•	1250			-1-		1255			U 1	Cly	
												•				
ACA	CAA	GTC	CTC	CTG	GGA	AGG	GCT	AAT	GAA	GCG	AGG	САТ	GTC	ΔΔΔ	AGA	1728
			Leu													1720
1260			-		1265					1270				-ys	_ -	
										12/0	•				1275	

CTT CAC GTG GGA CCA TGC CGC TGA Leu His Val Gly Pro Cys Arg * 1280

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 584 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein :
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
- Cys Val Ala Glu Leu Ser Arg Glu Gly Pro Ala Pro Arg Pro Leu Pro 1 5 10 15
- Pro Ala Leu Leu Ala Pro Pro Leu Val Pro Gly Phe Leu Ala Pro Pro 20 25 30
- Ala Glu Pro Thr Gly Glu Pro Ala Ser Thr Pro Pro Pro Val Pro Asp 35 40 45
- Ala Gly Leu Gly Asp Leu Gly Leu Glu Pro Glu Gly Ile Ala Glu Gly 50 55 60
- Ser Ile Asp Asn Thr Val Val Val Ala Ser Glu Gln Asp Ser Glu Ile 65 70 75 80
- Val Val Gly Lys Glu Gln Ala Arg Ala Lys Val Thr Gln Ser Ile Val 85 90 95
- Phe Val Thr Gly Glu Ala Ser Pro Tyr Ala Lys Ser Gly Gly Leu Gly
 100 105 110
- Asp Val Cys Gly Ser Leu Pro Val Ala Leu Ala Ala Arg Gly His Arg 115 120 125
- Val Met Val Val Met Pro Arg Tyr Leu Asn Gly Thr Ser Asp Lys Asn 130 135 140
- Tyr Ala Asn Ala Phe Tyr Thr Glu Lys His Ile Arg Ile Pro Cys Phe 145 150 155 160

Gly	Gl _y	y Glu	ı His	165		Thi	: Phe	Ph∈	∍ His 170		туг	Arq	J Asp	175	val
Asp	Tr	o Val	. Phe 180		. Asp	His	Pro	Se:		His	arç	Pro	Gly 190		ı Leu
Tyr	Gly	7 Asp 195		Phe	Gly	Ala	Phe 200		/ Asp) Asn	Gln	Phe 205		Туг	Thr
Leu	Leu 210	Cya	Tyr	Ala	Ala	Cys 215		Ala	Pro	Leu	Ile 220		Glu	Leu	Gly
Gly 225		·Ile	Туг	Gly	Gln 230	Asn	Суз	Met	- Phe	Val 235		Asn	Asp	Trp	His 240
Ala	Ser	Leu	Val	Pro 245	Val	Leu	Leu	Ala	Ala 250		Tyr	Arg	Pro	Tyr 255	Gly
Val	Tyr	Lys	Asp 260	Ser	Arg	Ser	Ile	Leu 265		Ile	His	Asn	Leu 270	Ala	His
Gln	Gly	Val 275	Glu	Pro	Ala	Ser	Thr 280	Tyr	Pro	Asp	Leu	Gly 285	Leu	Pro	Pro
Glu	Trp 290	Tyr	Gly	Ala	Leu	Glu 295	Trp	Val	Phe	Pro	Glu 300	Trp	Ala	Arg	Arg
His 305	Ala	Leu	Asp	Lys	Gly 310	Glu	Ala	Val	Asn	Phe 315	Leu	Lys	Gly	Ala	Val 320
Val	Thr	Ala	Asp	Arg 325	Ile	Val	Thr	Val	Ser 330	Lys	Gly	Tyr	Ser	Trp 335	Glu
Val	Thr	Thr	Ala 340	Glu	Gly	Gly	Gln	Gly 345	Leu	Asn	Glu	Leu	Leu 350	Ser	Ser
Arg	Lys	Ser 355	Val	Leu	Asn	Gly	Ile 360	Val	Asn	Gly	Ile	Asp 365	Ile	Asn	Asp
Trp	Asn 370	Pro	Ala	Thr		Lys 375	Суз	Ile	Pro		His 380	Tyr	Ser	Val	Asp
Asp :	Leu	Ser	Gly :		Ala 1 390	Lys	Cys	Lys		Ala 395	Leu	Gln	Lys	Glu	Leu 400

- Gly Leu Pro Ile Arg Pro Asp Val Pro Leu Ile Gly Phe Ile Gly Arg
 405 410 415
- Leu Asp Tyr Gln Lys Gly Ile Asp Leu Ile Gln Leu Ile Ile Pro Asp 420 425 430
- Leu Met Arg Glu Asp Val Gln Phe Val Met Leu Gly Ser Gly Asp Pro 435 440 445
- Glu Leu Glu Asp Trp Met Arg Ser Thr Glu Ser Ile Phe Lys Asp Lys 450 455 460
- Phe Arg Gly Trp Val Gly Phe Ser Val Pro Val Ser His Arg Ile Thr 465 470 475 480
- Ala Gly Cys Asp Ile Leu Leu Met Pro Ser Arg Phe Glu Pro Cys Gly 485 490 495
- Leu Asn Gln Leu Tyr Ala Met Gln Tyr Gly Thr Val Pro Val Val His 500 505 510
- Ala Thr Gly Gly Leu Arg Asp Thr Val Glu Asn Phe Asn Pro Phe Gly 515 520 520
- Glu Asn Gly Glu Gln Gly Thr Gly Trp Ala Phe Ala Pro Leu Thr Thr 530 540
- Glu Asn Met Phe Val Asp Ile Ala Asn Cys Asn Ile Tyr Ile Gln Gly
 545 550 555 560
- Thr Gln Val Leu Gly Arg Ala Asn Glu Ala Arg His Val Lys Arg
 565 570 575

Leu His Val Gly Pro Cys Arg * 580

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2725 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: not relevant
- (ii) MOLECULE TYPE: mRNA

(vi) ORIGINAL SOURCE:																
(A) ORGANISM: Zea mays																
	(iv) FEATURE.															
•	(ix) FEATURE: (A) NAME/KEY: sig_peptide															
									9							
			(B) 1	LOCA	rion	91	26	4								
	13.	د ۱ کا د	בו זות אם													
	(1.		EATUI		/VEV.				_							
			(A) 1 (B) I				_		€							
			(-, -			. 20.	2	,	•							
	(ix) FEATURE:															
	(A) NAME/KEY: CDS															
	(B) LOCATION: 912490															
(=, ===================================																
	(xi	.) SE	EQUEN	ICE D	ESCF	IPTI	ON:	SEQ	ID N	10:14	:					
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:																
GGCCCAGAGC AGACCCGGAT TTCGCTCTTG CGGTCGCTGG GGTTTTAGCA TTGGCTGATC															60	
															114	
Met Ala Phe Arg Val Ser Gly Ala																
- 58																
GTG	CTC	GGT	GGG	GCC	GTA	AGG	GCT	ccc	CGA	CTC	ACC	GGC	GGC	GGG	GAG	162
Val	Leu	Gly	Gly	Ala	Val	Arg	Ala	Pro	Arg	Leu	Thr	Glv	Glv	Glv	Glu	102
-50					-45					-40		•	1	1	-35	
GGT	AGT	CTA	GTC	TTC	CGG	CAC	ACC	GGC	CTC	TTC	TTA	ACT	CGG	GGT	GCT	210
Gly	Ser	Leu	Val	Phe	Arg	His	Thr	Gly	Leu	Phe	Leu	Thr	Arg	Gly	Ala	
				-30					-25					-20		
CGA	CTT	CCA	mc m													
Ara	Val	GUA	Cve	Sor	Clv	ACG	CAC	GGG	GCC	ATG	CGC	GCG	GCG	GCC	GCG	258
9	, 42	O.L.y	-15	Ser	Gry	THE	nis	-10	Ala	Met	Arg	Ala		Ala	Ala	
								-10			•		-5			
GCC	AGG	AAG	GCG	GTC	ATG	GTT	CCT	GAG	GGC	GAG	ልል ጥ	GAT	GGC	CTC	CCD	306
Ala	Arg	Lys	Ala	Val	Met	Val	Pro	Glu	Gly	Glu	Asn	Asp	Glv	Leu	Ala	306
		1				5			-		10		1			
TCA	AGG	GCT	GAC	TCG	GCT	CAA	TTC	CAG	TCG	GAT	GAA	CTG	GAG	GTA	CCA	354
Ser	Arg	Ala	Asp	Ser	Ala	Gln	Phe	Gln	Ser	Asp	Glu	Leu	Glu	Val	Pro	
15					20					25					30	

(iii) HYPOTHETICAL: NO

GA	C AT	T TC	T GA	A GA	G AC	A ACC	G TG	C GG	T GC	T GG	T GT	G GC	r ga	T GC	T CAA	402
As	p Il	e Se	r Gl	u Gl	u Th	r Thi	с Су	s Gl	y Al	a Gl	y Va	1 Al	a As	p Al	a Gln	-
				3	5				4	0				4	5	
GC	C TT	G AA	C AG	A GT	T CG	A GTG	GT	CCC	c cc	A CC	A AG	C GA	GG.	A CA	A AAA	450
Al	a Le	u As	n Ar	g Va	l Ar	y Val	. Vai	l Pro	o Pro	o Pro	o Se	r Asj	G1	y Gl	n Lys	
	-		5	0				5	5				6	0		
3.00																
T1.	A TT	C CA	G AT	r GA	C CC	ATG	TTC	G CA	A GG	C TA	r aa	G TAC	CA'	r ct	r gag	498
110	= Pn			e Ası	Pro	Met			ı Gly	у Ту	c Ly	s Tyr	Hi	s Le	ı Glu	
		6	5				70)				75	i			
TAT	r cc(2 ጥል	C ACC	- CTC	י היא ה											
															CAT	546
-1-	80		. Jei	. Let	LYL	Arg 85		ITIE	Arc	, Ser			Ası	Glu	His	
	•	•				03					90)				
GAA	GG?	A GGG	TTO	GAA	GCC	TTC	TCC	י רכיד	י אכיד	י מידי	י כאכ		mma		TTT	
Glu	Gly	Gly	/ Leu	Glu	Ala	Phe	Ser	Aro	201	. T.T.T	Clu	, AAG	חשר	GGE	Phe	594
95	;	_			100			9	001	105		ггуз	FILE	: GI		
										103					110	
AAT	GCC	AGO	GCG	GAA	GGT	ATC	ACA	TAT	CGA	GAA	TGG	: ርርጥ	CCT	' cca	GCA	642
Asn	Ala	Ser	Ala	Glu	Gly	Ile	Thr	Tyr	Ara	Glu	Tro	Ala	Pro	GGA	Ala	642
				115				•	120					125		
														123		
TTT	TCT	GCA	GCA	TTG	GTG	GGT	GAC	GTC	AAC	AAC	TGG	GAT	CCA	AAT	GCA	690
Phe	Ser	Ala	Ala	Leu	Val	Gly	Asp	Val	Asn	Asn	Trp	Asp	Pro	Asn	Ala	
			130					135					140			
GAT	CGT	ATG	AGC	AAA	AAT	GAG	TTT	GGT	GTT	TGG	GAA	ATT	TTT	CTG	CCT	738
Asp	Arg	Met	Ser	Lys	Asn	Glu	Phe	Gly	Val	Trp	Glu	Ile	Phe	Leu	Pro	
		145					150					155				
220	ת א	663	~~~													
Asn	Acn	Ala	GAT	GGT	ACA	TCA	CCT	ATT	CCT	CAT	GGA	TCT	CGT	GTA	AAG	786
non	160	nia	Asp	GIA	Thr	Ser	Pro	Ile	Pro	His		Ser	Arg	Val	Lys	
	100					165					170					
GTG	AGA	ATG	GAT	ልሮጥ	CCA	TCA	ccc	202	220							
Val	Ara	Met	Asp	Thr	Pro	Ser	GUG	TIO	AAG	GAT	TCA	ATT	CCA	GCC	TGG -	834
175	_				180		- 1	116	Lys	185	Ser	116	PFO	Ala	-	
										103					190	
ATC	AAG	TAC	TCA	GTG	CAG	GCC	CCA	GGA	GAA	ATA	CCA	тат	GAT	GGG	Δ·ሞ·ሞ	999
Ile	Lys	Tyr	Ser	Val	Gln	Ala :	Pro	Gly	Glu	Ile	Pro	Tvr	Asn	Glv	Lle	882
				195					200	- =		-1-	- P	205	**6	
														200		
TAT	TAT	GAT	CCT	CCT	GAA	GAG (GTA .	AAG	TAT	GTG	TTC	AGG	CAT	GCG	CAA	930
															J	730

ту	r T	yr E	Asp	Pro 210		o Gl	u Gl	lu Va		ys T LS	yr	Val	. Phe	e Ar	g Hi 22		.a	Gln		· ·
cc	T A2	AA C	:GA	CCA	. בב	ል ጥሮ	ል ጥፕ	G CG	יר אי	ים כי	7 CP									
Pr	o L	rs A	rg	Pro	Lys	s Se:	r Le	u Ar	g Il	.e T	vr (GAA Glu	ACA Thr	A CA:	r GT = V=	C GG	A .	ATG		978
		2	25		Ī			23		•	<i>7</i> - ·		****	2:3:		I GI	·Y	met		
AG	ፐ ልር		CC	~ n n	000		~													
Se.	r Se	r P	ro	GAA Glu	Pro	AAC	s AT s Il	A AA e As	n Th	A T	AT (GTA Vəl	AAC	TT	AG	G GA	T	GAA		1026
	24	0		*		-2	24			;	Y - '	AGI	250		Ar	J AS	p (ilu		
GT(C CT	C C	CA I	AGA	ATA	AAA	AA.	A CT	T GG	A, TA	AC A	AAT	GCA	GTG	CAZ	A AT	A A	ATG		1074
259	- De 5	u P	20 /	arg	116	260		s Le	u Gl	у ту		Asn 265	Ala	Val	Gli	ı Ile				
											4	.03					2	170	•	
GC	A AT	C C	AA (GAG	CAC	TCA	TA:	r TA	r gg.	A AG	C I	TT	GGA	TAC	CAT	GT	A A	CT		1122
Ala	a Il	e G	ln (Glu	His	Ser	Ту	r Ty	r Gl	y Se	r P	he	Gly	Tyr	His	Va:	1 1	'hr		
					275					28	0					289	5			
AAI	TT	r Ti	TT G	GCG	CCA	AGT	AGT	r cgi	r TT	r gg	та	cc	CCA	GAA	САТ	ጥጥረ	מ ב	3.C		1120
Asn	Phe	e Pł	ne A	Ala	Pro	Ser	Ser	Arg	g Phe	e Gl	уТ	hr	Pro	Glu	Asp	Leu	ı L	ys		1170
				90					295						300			-		
TCT	TTC	AI	T G	AT	AGA	GCA	CAT	GAG	י רייי	r cc	ጥ ጥ	TC	Cm 3				_			
Ser	Le	ıIl	e A	sp	Arg	Ala	His	Glu	Let	. GG	y L	eu	Leu	Val	Len	ATG	G · A	AT SD		1218
		30						310			•			315	204	****		35		
GTG	CTT	. Ca	ጥ አ	CT.	C N m	666														
Val	Val	. Hi	s S	er :	His	Ala	Ser	AGT Ser	AAT Asn	AC'	r C	TG	GAT	GGG	TTG	AAT	G G	GT		1266
	320	,					325						330	GTÅ	Leu	Asn	. G	тУ		
mmm.	63.				_															
Phe	Asp	GG G1	TA: VT:	CA (GAT	ACA	CAT	TAC	TTT	CAC	AC	GT (GGT	CCA	CGT	GGC	C	ΑT		1314
335		-	y +:	111 1	raħ	340	nis	Tyr	Pne	HIS	3 Se		Gly	Pro	Arg	Gly				
																		50		
CAC	TGG	AT	G TO	GG (GAT	TCT	CGC	CTA	TTT	AAC	TA	AT (GG	AAC	TGG	GAA	G7	T.		1362
His	Trp	Me	t T		Asp 355	Ser	Arg	Leu	Phe			r C	Gly	Asn	Trp	Glu	Va	1		
				-	223					360)					365				
TTA	AGA	TT	r ci	rr c	CTC	TCC	AAT	GCT	AGA	TGG	TG	G C	TC (GAG	GAA	TAT	AA	.G		1410
Leu	Arg	Phe	₽ Le	eu I	Leu	Ser	Asn	Ala	Arg	Trp	Tr	p I	.eu (Glu	Glu	Tyr	Ly	s		- ·- ·
			37	70					375						380					
TTT	GAT	GG1	TI	c c	GT '	TTT	GAT	GGT	GTG	ACC	ፓር	C A	.тс ^з	ልጥር ነ	רמר	א כייי	د.	c		1450
Phe	Asp	Gly	Ph	e A	rg 1	Phe	Asp	Gly	Val	Thr	Se	r M	let 1	Met '	Tyr	Thr	Hi	s		1458

CAC	GGA	TT	A CAA	GTA	ACA	TTT	ACG	GGG	AAC	TTC	AAT	GAG	TAI	TTT	' GGC	1506
His			Gln	Val	Thr	Phe	Thr	Gly	Asn	Phe	Ası	Glu	Tyr	Phe	Gly	
	400					405					410)-				•
TTI	GCC	ACC	GAT	GTA	GAT	GCA	GTG	GTT	TAC	TTG	ATO	CTG	GTA	AAT	GAT	1554
															Asp	1334
415					420					425					430	
CTA	ATT	CAT	GGA	CTT	TAT	CCT	GAG	GCT	GTA	ACC	ATI	GGT	GAA	GAT	GTT	1602
Leu	Ile	His	Gly	Leu	Tyr	Pro	Glu	Ala	Val	Thr	Ile	Gly	Glu	Asp	Val	
				435					440					445		
AGT	GGA	ATG	CCT	ACA	TTT	GCC	CTT	CCT	GTT	CAC	GAT	GGT	GGG	GTA	GGT	1650
Ser	Gly	Met	Pro	Thr	Phe	Ala	Leu	Pro	Val	His	Asp	Gly	Gly	Val	Gly	
			450					455					460			
			CGG													1698
Phe	Asp		Arg	Met	His	Met	Ala	Val	Ala	Asp	Lys	Trp	Ile	Asp	Leu	
		465					470					475				
			AGT													1746
Leu		Gln	Ser	Asp	Glu	Thr	Trp	Lys	Met	Gly	Asp	Ile	Val	His	Thr	
	480					485					490					
			AGG													1794
	Thr	Asn	Arg	Arg		Leu	Glu	Lys	Cys	Val	Thr	Tyr	Ala	Glu	Ser	
495					500					505					510	
			GCA													1842
His	Asp	Gln	Ala		Val	Gly	Asp	Lys	Thr	Ile	Ala	Phe	Trp	Leu	Met	
				515					520					525		
			ATG													1890
Asp	Lys	Asp	Met	Tyr	Asp	Phe	Met	Ala	Leu	Asp	Arg	Pro	Ser	Thr	Pro	•
			530					535					540			
			CGT													1938
Thr			Arg	Gly	Ile	Ala	Leu	His	Lys	Met	Ile	Arg	Leu	Ile	Thr	
		545					550					555				
			GGA													1986
Met		Leu	Gly	Gly	Glu	Gly	Tyr	Leu	Asn	Phe	Met	Gly	Asn	Glu	Phe	
	560					565					570					

003																
GGA	CA'	r cc	T GA	A TG	G AI	A GA	T TT	T CC	A AG	A GG	r ccc	G CA	A AG	A CI	T CC	A 2034
Gly	His	3 Pr	o G1	u Tr	p Il	e As	p Ph	e Pro	o Are	g Gly	y Pro	G1:	n Ar	g Le	u Pro	200,4
575					58					589				-	590	
AGT	GGT	r aa	G TT	T AT	тсс	A GG	G AA	r aac	: AA(7 AG1	ר תיתים		~			
Ser	Gly	, Ly	s Ph	e Il	e Pr	o Gl	y Ası	n Asr	Ası	. Ser	Tyr	. GAI	- AAA	TG	T CG	. 2082 -
				59		•			600		1-		, rå:	60 60	-	J
663																
Ara	AGA	TT'	I GA	C CT	G GG	r GA1	GC	A GAC	TAT	CTI	' AGG	TAT	CAT	GG	r Atc	2130
9	nry	F 110	= AS) 610	o 5 re	u GI	y Asp) Ala	Asp 615		Leu	Arg	Tyr			y Met	:
,				_				013					620)		
CAA	GAG	TT	r GAT	CAC	G GC	A ATG	CAA	CAT	CTT	GAG	CAA	AAA	TAT	' GAZ	A TTC	2178
Gln	Glu	Phe	e Ası	, Gl	n Ala	a Met	Gln	His	Leu	Glu	Gln	Lys	Tyr	Glu	ı Phe	2170
		625	5				630	l				635				
ATG	ACA	тст	GAT	י רשנ	י ראר	TAT	ስ ጥጥ	TCC.	000							
Met	Thr	Ser	Asp	His	Glr	Tyr	Ile	Ser	Ara	AAA Luc	CAT	GAG	GAG	GAT	`AAG	2226
	640		•			645			••• 9	Lys	650	GIU	Gru	AST	rys	
GTG	ATT	GTG	TTC	GAA	AAG	GGA	GAT	TTG	GTA	TTT	GTG	TTC	AAC	TTC	CAC	2274
055	Ile	Val	Phe	Glu		Gly	Asp	Leu	Val	Phe	Val	Phe	Asn	Phe	His	
033					660					665					670	
TGC A	AAC	AAC	AGC	TAT	TTT	GAC	TAC	CGT	ATT	GGT	ጥርጥ	CGA	AAC	CCT	ccc	0200
Cys A	Asn	Asn	Ser	Tyr	Phe	Asp	Tyr	Arg	Ile	Gly	Cys	Ara	Lvs	Pro	Glv	2322
				675					680		_	•	•	685	1	
CTC T	3 m															
GTG T Val T	'vr	AAG	GTG Val	GTC	TTG	GAC	TCC	GAC	GCT	GGA	CTA	TTT	GGT	GGA	TTT	2370
Val T		Dy 3	690	Val	reu	Asp	ser	Asp 695	Ala	Gly	Leu	Phe		Gly	Phe	
								093					700			
AGC A	.GG	ATC	CAT	CAC	GCA	GCC	GAG	CAC	TTC	ACC	GCC (GAC	TGT	TCG	CAT	2418
Ser A	rg	Ile	His	His	Ala	Ala	Glu	His	Phe	Thr .	Ala	Asp	Cys	Ser	His	2.20
		705					710				•	715				
GAT A	AT 2	AGG	CCA	TAT	TCA	TTC	TCG	ርጥጥ '	ייבים	מים ב	CO2 -					
Asp A	sn 2	Arg	Pro	Tyr	Ser	Phe	Ser	Val 1	Tyr '	Thr 1	Pro 9	nGC Ser	AGA . Ara '	ACA Th∽	TGT	2466
7:	20					725			- 4 -		730 730		nr g	LIIL	CAR	
3ma -													•			
GTC GT	rc 1	TAT	GCT	CCA	GTG	GAG '	TGA :	TAGCO	GGG:	ra ct	rcgti	GCT	G CG	CGGC	ATGT	2520
al Va	-1]	yr	wra		Val 740	Glu	*									
35																

CI	'ACAA	TAAG	GTT	CTGA	TAC	TTTA	ATCG	AT G	CTGG	AAAG	c cc	ATGC	ATCT	CGC	TGCGTTG
TC	CTCT	CTAT	ATA	TATA	AGA	CCTT	CAAG	GT G	TCAA	TTAA	A CA	TAGA	GTTT	TCG	TTTTTCG
CT	TTCC	TAAA	AAA	AAAA	AAA I	AAAA	A								
40															
(2) IN	FORM	ATIO!	N FO	R SE	Q ID	NO:	15:							
		(i)			e chi ength					is					
					(PE: OPOLO				•						
		(ii)			E TYP										
					DES				.O TE	NO.	15.				
17 - 4															
-58	: Ala	l Phe	-55	y Val	. Ser	Gly	Ala	-50		Gly	Gly	Ala	Val -45		Ala
Pro	Arg	Leu -40	Thr	Gly	Gly	Gly			Ser	Leu	Val			His	Thr
							-35					-30			
Gly	Leu -25	Phe	Leu	Thr	Arg	Gly -20		Arg	Val	Gly	Cys -15	Ser	Gly	Thr	His
Gly	Ala	Met	Arg	Ala		Ala	Ala	Ala	Arg	Lys	Ala	Val	Met	Val	Pro
					- 5					1				5	
Glu	Gly	Glu	Asn 10	Asp	Gly	Leu	Ala	Ser 15	Arg	Ala	Asp	Ser	Ala 20	Gln	Phe
Gln	Ser	Asp	Glu	Leu	Glu	Val	Pro	Asp	Ile	Ser	Glu	Glu	Thr	Thr	Cys
		25					30					35			-
Gly	Ala 40	Gly	Val	Ala	Asp	Ala 45	Gln	Ala	Leu	Asn	Arg 50	Val	Arg	Val	Val
Pro	Pro	Pro	Ser	Asp	Gly	Gln	Lys	Ile	Phe	Gln	Ile	Asp	Pro	Met	Leu
55					60					65					70
Gln	Gly	Tyr	Lys	Tyr 75	His	Leu	Glu	Tyr	Arg 80	Tyr	Ser	Leu	Tyr	Arg 85	Arg

270C

Ile Arg Ser Asp Ile Asp Glu His Glu Gly Gly Leu Glu Ala Phe Ser Arg Ser Tyr Glu Lys Phe Gly Phe Asn Ala Ser Ala Glu Gly Ile Thr Tyr Arg Glu Trp Ala Pro Gly Ala Phe Ser Ala Ala Leu Val Gly Asp Val Asn Asn Trp Asp Pro Asn Ala Asp Arg Met Ser Lys Asn Glu Phe Gly Val Trp Glu Ile Phe Leu Pro Asn Asn Ala Asp Gly Thr Ser Pro Ile Pro His Gly Ser Arg Val Lys Val Arg Met Asp Thr Pro Ser Gly Ile Lys Asp Ser Ile Pro Ala Trp Ile Lys Tyr Ser Val Gln Ala Pro Gly Glu Ile Pro Tyr Asp Gly Ile Tyr Tyr Asp Pro Pro Glu Glu Val Lys Tyr Val Phe Arg His Ala Gln Pro Lys Arg Pro Lys Ser Leu Arg Ile Tyr Glu Thr His Val Gly Met Ser Ser Pro Glu Pro Lys Ile Asn Thr Tyr Val Asn Phe Arg Asp Glu Val Leu Pro Arg Ile Lys Lys Leu Gly Tyr Asn Ala Val Gln Ile Met Ala Ile Gln Glu His Ser Tyr Tyr Gly Ser Phe Gly Tyr His Val Thr Asn Phe Phe Ala Pro Ser Ser Arg Phe Gly Thr Pro Glu Asp Leu Lys Ser Leu Ile Asp Arg Ala His Glu Leu Gly Leu Leu Val Leu Met Asp Val Val His Ser His Ala Ser Ser

- Asn Thr Leu Asp Gly Leu Asn Gly Phe Asp Gly Thr Asp Thr His Tyr 330 335 340
- Phe His Ser Gly Pro Arg Gly His His Trp Met Trp Asp Ser Arg Leu 345 350 355
- Phe Asn Tyr Gly Asn Trp Glu Val Leu Arg Phe Leu Leu Ser Asn Ala 360 365 370
- Arg Trp Trp Leu Glu Glu Tyr Lys Phe Asp Gly Phe Arg Phe Asp Gly 375 380 385 390
- Val Thr Ser Met Met Tyr Thr His His Gly Leu Gln Val Thr Phe Thr 395 400 405
- Gly Asn Phe Asn Glu Tyr Phe Gly Phe Ala Thr Asp Val Asp Ala Val 410 415 420
- Val Tyr Leu Met Leu Val Asn Asp Leu Ile His Gly Leu Tyr Pro Glu 425 430 430
- Ala Val Thr Ile Gly Glu Asp Val Ser Gly Met Pro Thr Phe Ala Leu 440 445 450
- Pro Val His Asp Gly Gly Val Gly Phe Asp Tyr Arg Met His Met Ala 455 460 460 465 465 465
- Val Ala Asp Lys Trp Ile Asp Leu Leu Lys Gln Ser Asp Glu Thr Trp
 475 480 485
- Lys Met Gly Asp Ile Val His Thr Leu Thr Asn Arg Arg Trp Leu Glu 490 495 500
- Lys Cys Val Thr Tyr Ala Glu Ser His Asp Gln Ala Leu Val Gly Asp 505 510 515
- Lys Thr Ile Ala Phe Trp Leu Met Asp Lys Asp Met Tyr Asp Phe Met 520 525 530
- Ala Leu Asp Arg Pro Ser Thr Pro Thr Ile Asp Arg Gly Ile Ala Leu 535 545 545 550
- His Lys Met Ile Arg Leu Ile Thr Met Gly Leu Gly Gly Glu Gly Tyr
 555 560 565

- Leu Asn Phe Met Gly Asn Glu Phe Gly His Pro Glu Trp Ile Asp Phe 570 575 580
- Pro Arg Gly Pro Gln Arg Leu Pro Ser Gly Lys Phe Ile Pro Gly Asn 585 590 595
- Asn Asn Ser Tyr Asp Lys Cys Arg Arg Arg Phe Asp Leu Gly Asp Ala 600 605 610
- Asp Tyr Leu Arg Tyr His Gly Met Gln Glu Phe Asp Gln Ala Met Gln 615 620 625 630
- His Leu Glu Gln Lys Tyr Glu Phe Met Thr Ser Asp His Gln Tyr Ile
 635 640 645
- Ser Arg Lys His Glu Glu Asp Lys Val Ile Val Phe Glu Lys Gly Asp 650 660
- Leu Val Phe Val Phe Asn Phe His Cys Asn Asn Ser Tyr Phe Asp Tyr 665 670 675
- Arg Ile Gly Cys Arg Lys Pro Gly Val Tyr Lys Val Val Leu Asp Ser 680 685 690
- Asp Ala Gly Leu Phe Gly Gly Phe Ser Arg Ile His His Ala Ala Glu 695 700 705 710
- His Phe Thr Ala Asp Cys Ser His Asp Asn Arg Pro Tyr Ser Phe Ser 715 720 725
- Val Tyr Thr Pro Ser Arg Thr Cys Val Val Tyr Ala Pro Val Glu * 730 735 740

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2763 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: not relevant
- (ii) MOLECULE TYPE: mRNA
- (iii) HYPOTHETICAL: NO

(A) ORGANISM: Zea mays	
(ix) FEATURE:	
(A) NAME/KEY: transit_peptide	
(B) LOCATION: 2190	•
(ix) FEATURE:	
(A) NAME/KEY: mat_peptide	
(B) LOCATION: 1912467	
(ix) FEATURE:	
(A) NAME/KEY: CDS	
(B) LOCATION: 22470	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:	
G CTG TGC CTC GTG TCG CCC TCT TCC TCG CCG ACT CCG CTT CCG CCG	46
Leu Cys Leu Val Ser Pro Ser Ser Ser Pro Thr Pro Leu Pro Pro	
-63 -60 -55 -50	
CCG CGG CGC TCT CGC TCG CAT GCT GAT CGG GCG GCA CCG CCG GGG ATC	0.4
Pro Arg Arg Ser Arg Ser His Ala Asp Arg Ala Ala Pro Pro Gly Ile	94
-45 -40 -35	
GCG GGT GGC GGC AAT CTC GGG CTC AGT AGT AGT	
GCG GGT GGC GGC AAT GTG CGC CTG AGT GTG TTG TCT GTC CAG TGC AAG Ala Gly Gly Asn Val Arg Leu Ser Val Leu Ser Val Gln Cys Lys	142
- - (()	
-20	
GCT CGC CGG TCA GGG GTG CGG AAG GTC AAG AGC AAA TTC GCC ACT GCA	190
Ala Arg Arg Ser Gly Val Arg Lys Val Lys Ser Lys Phe Ala Thr Ala	
-10 -5	
GCT ACT GTG CAA GAA GAT AAA ACT ATG GCA ACT GCC AAA GGC GAT GTC	220
Ala Thr Val Gln Glu Asp Lys Thr Met Ala Thr Ala Lys Gly Asp Val	238
1 5 10 15	• .
GAC CAT CTC CCC ATA TAC CAC CTC CAC CTC	
GAC CAT CTC CCC ATA TAC GAC CTG GAC CCC AAG CTG GAG ATA TTC AAG Asp His Leu Pro Ile Tyr Asp Leu Asp Pro Lys Leu Glu Ile Phe Lys	286
20	
30	
AC CAT TTC AGG TAC CGG ATG AAA AGA TTC CTA GAG CAG AAA GGA TCA	334
asp his Phe Arg Tyr Arg Met Lys Arg Phe Leu Glu Gln Lys Gly Ser	
35 40 45	

(vi) ORIGINAL SOURCE:

ATT	GAA	A GAZ	A AA'	r GAC	G GGA	a acr	г ст	T C	א מ	~m .	~~~								
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	30	,				55	5					60)						
AAA -	TTT	' GGG	ATI	TAA T	ACA	AAT	' GA	G GA	T GO	GA A	ACT	GTA	TA:	r co	T G	AA	TGG		430
Lys	Phe	Gly	' Ile	Asn	Thr	Asn	Gl	u As	p G	ly 1	Chr	Val	Tv	r Ai	a G	1,,	7~~		430
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GCA	CCT	GCT	GCG	CAG	GAG	GCA	GAG	- CT	יי איי	ייתי ה				_					
Ala	Pro	Ala	Ala	Gln	Glu	212	Cl	, r.		. 1 6	GT	GAC	TTC	: AA	T G	AC	TGG	•	478
				85	Glu	ura	GI	ı Le			TÀ	Asp	Phe	As	n A	sp	Trp		
				0.3					9	0					9	95			
ממב	CCT	CCN							_										
yen	Class	GCA	AAC	CAT	AAG	ATG	GAG	; AA	G. GA	TA	AA	TTT	GGT	GT	T T	3G	TCG	5	526
vall	GIĀ	ATA	Asn	His	Lys	Met	Glu	Ly	s As	p L	ys :	Phe	Gly	Va	1 .Tz	: p	Ser		
			100					10	5					11		•			
ATC	AAA	ATT	GAC	CAT	GTC	AAA	GGG	AAA	A CC'	T G	CC 2	ATC	ССТ	CA	~ ^ ^	Tr.	TCC	_	
Ile	Lys	Ile	Asp	His	Val	Lys	Gly	Lvs	s Pro	o A	la i	Tla	Dec	UZ.	- 1	ιı	rcc	5	74
		115		•		-	120	-2						nı:	s As	n	Ser		
													125						
AAG	GTT	AAA	ттт	CGC	ጥጥ	وشات	C 3 M												
Lvs	Val	Luc	Pho	7	TTT	CIM	CAT	GGT	GG	A GI	CA I	rgg	GTT	GAT	CG	T Z	TTA	6	22
Lys	130	ny s	rne	Arg	Pne	Leu	His	Gly	. Gl ³	/ Va	l I	rp	Val	Asp	Ar	g :	Ile		
	130					135					1	40							
222																			
CCA (GCA '	TTG	ATT	CGT	TAT (GCG	ACT	GTT	GAT	GC	C T	CT	AAA	TTT	GG	A C	CT	6	70
FLO A	Alai	Leu	Ile	Arg	Tyr :	Ala	Thr	Val	Asp	Al	a S	er 1	Lvs	Phe	G1	v Z	lla	· ·	70
145					150					15			4 -				.60		
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CCC 1	CAT (GAT (GGT (GTT (CAT 1	rgg (GAT	ССТ	ССТ	GC'	т т	CT C		100					
Pro 1	Cyr A	Asp (Gly '	Val I	His 1	ro /	Asp	Pro	Dro	וג	- 1'		AA.	AGG	TAC) A	CA	7:	18
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									170						175	5			
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TTT A	ve u	ii. T		-	CT 1	CA F	AAG	CCT	GCT	GC:	r co	CA C	GT	ATC	TAT	' G	AA	76	6
Phe L	iya n	115 1	rio F	arg E	ro s	er I	ys	Pro	Ala	Ala	a Pr	ro A	rg	Ile	Tyr	G	lu		
		1	.80					185						190					
CCC -		_																	
GCC C	AT G	TA G	GT A	TG A	GT G	GT G	AA I	AAG	CCA	GCA	GI	A A	GC I	ACA	TAT	A	GG	81	4
Ala H	is V	al G	ly M	let S	er G	ly G	lu 1	Lys	Pro	Ala	. Va	al S	er 1	Chr	Tur	Δ,	ra	01	7
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GAA T	TT G	CA G	AC A	AT G	TG T	TG C	CA C	GC	בידב	רכ ז	cc	7 .	. m -						
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	-				24				ic A		aı s 50	er	ser	Ar	g Se:	r Gl 25	_	Thr		
CC	A GA	AG (GAC	CTO	C AA	A TA	T CI	T GI	T G	AT A	AG G	CA	CAC	AG	TT	G GG	T 1	TTG		1006
PE	0 G1	.u F	Asp	Let 260	ı Ly	s Ty	r Le	eu Va			ys A	la	His	Ser	: Le	ı Gl	уІ	Leu		
				200	,				26	5		*			270)				
CG.	A GI	T C	TG	ATG	GA'	T GT	T GT	C CA	T AG	C C	AT G	CA	AGT	Аат	י אמי	ר כיד	C 2	(C)		1054
Ar	g Va	1 [eu	Met	As	p Va	l Va	l Hi	s Se	eri. H	is A	la	Ser	Asn	Asr	. Va	17	hr.		1054
		2	75					28						285						
GA'	r cc	ጥ ጥ	מידי	ייבג		י מיחי	T (3	m .cm						•						
Ası	- G1	y L	eu	Asn	Gl	· TV	r GA	T GT p Va	T GG	A C	AA A	GC .	ACC	CAA	GAG	TC	CI	'AT		1102
	29	υ O				-1	29		- 01	.y G.	LII 30		300	GIN	Glu	Se	r T	yr		
TTI	CA	r G	CG ,	GGA	GA1	' AG	A GG	T TA	r ca	T AP	LA C	rr :	TGG	GAT	AGT	CGC	3 C	TG		1150
305	; H1:	s A	la	Gly	Asp			у Ту:	r Hi	s Ly			Trp	Asp	Ser	Arg	J L	eu		
						310	,				31	L5					3	20		
TTC	AA	T	AT	GCT	AAC	TGG	GAC	G GTA	A TT	A AG	G TI	T C	CTT	СТТ	TICT	A A C	٠ ,	TC		1100
Phe	Ası	ı Ty	yr.	Ala	Asn	Trp	Glu	ı Val	Le	u Ar	g Ph	ie I	Leu	Leu	Ser	Asn	L	eu		1198
					325					33						335				
AGA	TAT	· To	3G '	TTG	GAT	GAA	ጥጥር	ATC												
Arg	Tyr	Tr	p 1	Leu	Asp	Glu	Phe	Met	. Phe	. GA e As	i GG b Gl	v P	TC he	CGA	TTT	GAT	G	GA		1246
			:	340					345			. ·		nry	350	nsp	G.	гÀ		
CTT																				
Val	Thr	. TC	A A	ATG	CTG	TAT	CAT	CAC	CAT	GG	r at	C A	AT (GTG	GGG	TTT	AC	CT	1	1294
		35	5	.e.c	Leu	TÄL	nis	His		GL	y Il	e A		_	Gly	Phe	Th	ır		
									•				•	365						
GGA	AAC	TA	.c c	CAG	GAA	TAT	TTC	AGT	TTG	GAG	AC	A G	CT (GTG	GAT	GCA	GI	T'	1	.342
Gly	Asn 370	Ту	r G	ln	Glu	Tyr	Phe	Ser	Leu	Asp	Th	r A	la v	Val	Asp	Ala	Va	1		
	370						375					3	80							
GTT	TAC	AT	G A	TG	CTT	GCA	AAC	CAT	TTA	ATG	CAC	: A	AA r	TC '	ጥጥር፡	רכז	C 3	A	,	300
Val	Tyr	Me	t M	let :	Leu	Ala	Asn	His	Leu	Met	His	L	ys I	Leu :	Leu	Pro	GA Gl	u u	T	390
385				•		390					395						40			
GCA	АСТ	GT ^r	ם ק	ጥጥ /	3.Cm	מ מים	C 3 M	c==	m~-											
Ala	Thr	Va:	- G 1 V	al A	Ala	Glu	Asp	GTT Val	TCA	GGC G1.	ATG	CC	CG G	TC (CTT	TGC	CG	G	1	438
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															Ala	1486
			420					425		•		, – -	430			
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TTE	PIC	439 439		Trp) Ile	e Asp			Lys	Asn	Lys			Ser	Glu	
		43.	,				440	1				445				
TGG	TCC	ATC	G GGT	' GAA	ATA	GCG	CAT	ACI	TTG	ACT	AAC	AGG	AGA	тат	ACT	1582
			Gly													1302
	450					455		•:			460	•	,	- 4 -		
			ATC													1630
465		Cys	Ile	Ala			Glu	Ser	His	Asp	Gln	Ser	Ile	Val	Gly	
403					470					475	•				480	
GAC	AAA	ACT	ATT	GCA	TTT	СТС	CTG	ATG	GAC	AAG	GAA	እ <i>ጥር</i>	ma c	3 CM	600	1670
			Ile													1678
				485					490	-10			TYL	495	Gry	
														.,,		
			TTG													1726
Met	Ser	Asp	Leu	Gln	Pro	Ala	Ser	Pro	Thr	Ile	Asp	Arg	Gly	Ile	Ala	
			500					505					510			
CTC	CAA	A A C	ATC	יייים ע	CAC	mmc.	3.00	202								
Leu	Gln	Lvs	ATG Met	Ile	His	Phe	Tle	The	Mot	Ala	CTT	GGA	GGT	GAT	GGC	1774
		515					520	****	nec	nia	Leu	525	GIY	Asp	GIY	
												723				
			TTT													1822
Tyr		Asn	Phe	Met	Gly	Asn	Glu	Phe	Gly	His	Pro	Glu	Trp	Ile	Asp	
	530					535					540					
TTT	CCA	AGA	GAA	GGG	220	33 C	TCC	ACC	መለጥ	C 3 m						
Phe	Pro	Ara	Glu	Glv	Asn	Asn	Trn	Ser	TVF	GAT	AAA	TGC	AGA	CGA	CAG	1870
545				2	550				-1-	555	Буз	cys	Arg	Arg	560	,
															360	
TGG	AGC	CTT	GTG	GAC	ACT	GAT	CAC	TTG	CGG	TAC	AAG	TAC	ATG	AAT	GCG	1918
Trp	Ser	Leu	Val	Asp	Thr	Asp	His	Leu	Arg	Tyr	Lys	Tyr	Met	Asn	Ala	
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			GCG Ala													1966
		11	Ala 580		กอแ	ura		Asp 585	GIU	arg	rne			Leu	Ser	
													590			

TC Se	G TC. r Sé	A AA r Ly 59	s Gln	ATO	C GTO	C AGC	GAG Asi	Met	G AAC	C GAI Nasp	GAC	G GAA 1 Glu 605	Lys	GT1	ATT	2014
GT Va	C TT: l Phe 610	e Gl₁	A CGT 1 Arg	GGA Gly	A GAI	TTA Leu 615	Va.l	TT1	GTT Val	TTC Phe	AAT Asr	Phe	CAT His	CCC	AAG Lys	2062
AAZ Lys 625	5 Thr	TAC	C GAG	GGC	TAC Tyr 630	Lys	GTG Val	GGA Gly	Cys	GAT Asp 635	Leu	Pro	GGG Gly	AAA Lys	TAC Tyr 640	2110
AGA	A GTA	GCC Ala	CTG Leu	GAC Asp 645	Ser	GAT Asp	GCT Ala	CTG Leu	GTC Val 650	TTC Phe	GGT Gly	GGA Gly	CAT	GGA Gly 655	AGA Arg	2158
GTT Val	GGC Gly	CAC His	GAC Asp 660	GTG Val	GAT Asp	CAC His	TTC Phe	ACG Thr 665	TCG	CCT Pro	GAA Glu	GGG Gly	GTG Val 670	CCA Pro	GGG Gly	2206
GTG Val	CCC Pro	GAA Glu 675	ACG Thr	AAC Asn	TTC Phe	AAC Asn	AAC Asn 680	CGG Arg	CCG Pro	AAC Asn	TCG Ser	TTC Phe 685	AAA Lys	GTC Val	CTT Leu	2254
TCT Ser	CCG Pro 690	CCC Pro	CGC Arg	ACC Thr	TGT Cys	GTG Val 695	GCT Ala	TAT Tyr	TAC Tyr	CGT Arg	GTA Val 700	GAC Asp	GAA Glu	GCA Ala	GGG Gly	2302
GCT Ala 705	GGA Gly	CGA Arg	CGT Arg	CTT Leu	CAC His 710	GCG Ala	AAA Lys	GCA Ala	GAG Glu	ACA Thr 715	GGA Gly	AAG Lys	ACG Thr	TCT Ser	CCA Pro 720	2350
GCA Ala	GAG Glu	AGC Ser	ATC Ile	GAC Asp 725	GTC Val	AAA Lys	GCT Ala	TCC Ser	AGA Arg 730	GCT Ala	AGT Ser	AGC Ser	AAA Lys	GAA Glu 735	GAC Asp	2398
AAG Lys	GAG Glu	GCA Ala	ACG Thr 740	GCT Ala	GGT Gly	GGC /	Lys	AAG Lys 745	GGA Gly	TGG	AAG Lys	Phe :	GCG Ala 750	CGG Arg	CAG Gln	2446
CCA Pro	Ser	GAT Asp 755	CAA 6	GAT .	ACC .	Lys	rga * 760	AGCC	ACGA	GT C	CTTG	GTGA	G GA	CTGG	ACTG	2500
GCTG	CCGG	CG C	CCTG	rtag'	r ag	rccto	CTC	TAC	rgga(CTA C	GCCG	CCGC	rg g	CGCC	CTTGG	2560

AA	CGGT	CCTT	TCC	TGTA	GCT	TGCA	GGCG.	AC T	GG T G'	TCTC.	A TC	ACCG.	AGCA	GGC	AGGCACT
GC'	TTGT	ATAG	CTT	TTCT	AGA	ATAA'	TAAT	CA G	GGAT	GGAT	G GA	TGGT	GTGT	ATT	GGCTATC
TG	GCTA	GACG	TGC	ATGTO	GCC (CAGT'	rtgt:	AT G	TACA	GGAG	C AG	TTCC	CGTC	CAG	AATAAA
AA	AAAC:	TTGT	TGG	GGGT	TTT :	TTC									
(2)) INI	FORM	OITA	1 FOF	R SEÇ	Q ID	NO: 3	L7:							
		(i)	SEQU	JENCE	CHA	ARACI	CERIS	TICS	S:						
				A) LE					_	ls					
			(E	3) TY	PE:	amir	o ac	id							
			([) TC	POLC	GY:	line	ear							
	(ii)	MOLE	CULE	TYF	E: F	rote	in							
	(xi)	SEQU	ENCE	DES	CRIP	TION	: SE	Q ID	NO:	17:				
Leu	Cys	Leu	Val	Ser	Pro	Ser	Ser	Ser	Pro	Thr	Pro	Leu	Pro	Pro	Pro
-63			-60					- 55					-50		
Arg	Arg	Ser		Ser	His	Ala			Ala	Ala	Pro			Ile	Ala
		-43					-40					-35			
Gly	Gly -30		Asn	Val	Arg	Leu -25		Val	Leu	Ser	Val -20	Gln	Cys	Lys	Ala
3		_	_,								•				
-15	Arg	Ser	GLY	Val	Arg -10		Val	Lys	Ser		Phe	Ala	Thr	Ala	
					-10					-5					1
Thr	Val	Gln	Glu 5	Asp	Lys	Thr	Met	Ala 10	Thr	Ala	Lys	Gly	Asp 15	Val	Asp
His	Leu	Pro 20	Ile	Tyr	Asp	Leu		Pro	Lys	Leu	Glu		Phe	Lys	Asp
		20					25					30			
His	Phe 35	Arg	Tyr	Arg	Met	Lys 40	Arg	Phe	Leu	Glu	Gln 45	Lys	Gly	Ser	Ile
Glu	G1	A ~ -	C 1	C1:	C -	• -	a :	-		_	_				
50	GIU	ASN	GIU	Gly	Ser 55	red	Glu	Ser	Phe	Ser 60	Lys	Gly	Tyr	Leu	Lys 65

Phe Gly Ile Asn Thr Asn Glu Asp Gly Thr Val Tyr Arg Glu Trp Ala

Pro Ala Ala Glu Glu Ala Glu Leu Ile Gly Asp Phe Asn Asp Trp Asn Gly Ala Asn His Lys Met Glu Lys Asp Lys Phe Gly Val Trp Ser Ile

Lys Ile Asp His Val Lys Gly Lys Pro Ala Ile Pro His Asn Ser Lys

- Val Lys Phe Arg Phe Leu His Gly Gly Val Trp Val Asp Arg Ile Pro
- Ala Leu Ile Arg Tyr Ala Thr Val Asp Ala Ser Lys Phe Gly Ala Pro
- Tyr Asp Gly Val His Trp Asp Pro Pro Ala Ser Glu Arg Tyr Thr Phe
- Lys His Pro Arg Pro Ser Lys Pro Ala Ala Pro Arg Ile Tyr Glu Ala
- His Val Gly Met Ser Gly Glu Lys Pro Ala Val Ser Thr Tyr Arg Glu
- Phe Ala Asp Asn Val Leu Pro Arg Ile Arg Ala Asn Asn Tyr Asn Thr
- Val Gln Leu Met Ala Val Met Glu His Ser Tyr Tyr Ala Ser Phe Gly
- Tyr His Val Thr Asn Phe Phe Ala Val Ser Ser Arg Ser Gly Thr Pro
- Glu Asp Leu Lys Tyr Leu Val Asp Lys Ala His Ser Leu Gly Leu Arg
- Val Leu Met Asp Val Val His Ser His Ala Ser Asn Asn Val Thr Asp
- Gly Leu Asn Gly Tyr Asp Val Gly Gln Ser Thr Gln Glu Ser Tyr Phe
- His Ala Gly Asp Arg Gly Tyr His Lys Leu Trp Asp Ser Arg Leu Phe

Asn Tyr Ala Asn Trp Glu Val Leu Arg Phe Leu Leu Ser Asn Leu Arg 325 330 335

315

Tyr Trp Leu Asp Glu Phe Met Phe Asp Gly Phe Arg Phe Asp Gly Val

Thr Ser Met Leu Tyr His His His Gly Ile Asn Val Gly Phe Thr Gly 355 360 365

Asn Tyr Gln Glu Tyr Phe Ser Leu Asp Thr Ala Val Asp Ala Val Val 370 385

Tyr Met Met Leu Ala Asn His Leu Met His Lys Leu Leu Pro Glu Ala 390 395 400

Thr Val Val Ala Glu Asp Val Ser Gly Met Pro Val Leu Cys Arg Pro 405 410 415

Val Asp Glu Gly Gly Val Gly Phe Asp Tyr Arg Leu Ala Met Ala Ile 420 425 430

Pro Asp Arg Trp Ile Asp Tyr Leu Lys Asn Lys Asp Asp Ser Glu Trp 435 440 445

Ser Met Gly Glu Ile Ala His Thr Leu Thr Asn Arg Arg Tyr Thr Glu 450 455 460 465

Lys Cys Ile Ala Tyr Ala Glu Ser His Asp Gln Ser Ile Val Gly Asp 470 480

Lys Thr Ile Ala Phe Leu Leu Met Asp Lys Glu Met Tyr Thr Gly Met 485 490 495

Ser Asp Leu Gln Pro Ala Ser Pro Thr Ile Asp Arg Gly Ile Ala Leu 500 505 510

Gln Lys Met Ile His Phe Ile Thr Met Ala Leu Gly Gly Asp Gly Tyr 515 520 525

Leu Asn Phe Met Gly Asn Glu Phe Gly His Pro Glu Trp Ile Asp Phe 530 545

Pro Arg Glu Gly Asn Asn Trp Ser Tyr Asp Lys Cys Arg Arg Gln Trp

- Ser Leu Val Asp Thr Asp His Leu Arg Tyr Lys Tyr Met Asn Ala Phe 565 570 575
- Asp Gln Ala Met Asn Ala Leu Asp Glu Arg Phe Ser Phe Leu Ser Ser 580 585 590
- Ser Lys Gln Ile Val Ser Asp Met Asn Asp Glu Glu Lys Val Ile Val 595 600 605
- Phe Glu Arg Gly Asp Leu Val Phe Val Phe Asn Phe His Pro Lys Lys 610 615 620 625
- Thr Tyr Glu Gly Tyr Lys Val Gly Cys Asp Leu Pro Gly Lys Tyr Arg 630 635 640
- Val Ala Leu Asp Ser Asp Ala Leu Val Phe Gly Gly His Gly Arg Val 645 650 655
- Gly His Asp Val Asp His Phe Thr Ser Pro Glu Gly Val Pro Gly Val 660 665 670
- Pro Glu Thr Asn Phe Asn Asn Arg Pro Asn Ser Phe Lys Val Leu Ser 675 680 685
- Pro Pro Arg Thr Cys Val Ala Tyr Tyr Arg Val Asp Glu Ala Gly Ala 690 695 700 705
- Gly Arg Arg Leu His Ala Lys Ala Glu Thr Gly Lys Thr Ser Pro Ala 710 715 720
- Glu Ser Ile Asp Val Lys Ala Ser Arg Ala Ser Ser Lys Glu Asp Lys
 725 730 735
- Glu Ala Thr Ala Gly Gly Lys Lys Gly Trp Lys Phe Ala Arg Gln Pro
 740 745 750
- Ser Asp Gln Asp Thr Lys * 755 760
- (2) INFORMATION FOR SEQ ID NO:18:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 153 base pairs

(5) III . MUCIEIC ACIU	
(C) STRANDEDNESS: single	٠,
(D) TOPOLOGY: not relevant	
(ii) MOLECULE TYPE: cDNA to mRNA	
(iii) HYPOTHETICAL: NO	
(vi) ORIGINAL SOURCE:	
(A) ORGANISM: Zea mays	
(ix) FEATURE:	
(A) NAME/KEY: CDS	
(B) LOCATION: 1153	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:	
ATC CCC ACC CCC TCC TCC TCC	
ATG GCG ACG CCC TCG GCC GTG GGC GCC GCG TGC CTC CT	48
Met Ala Thr Pro Ser Ala Val Gly Ala Ala Cys Leu Leu Ala Arg	
765 . 770 775	
GCC GCC TGG CCG GCC GTC GGC GAC CGG GCG CGC CCG CGG AGG CTC	96
Ala Ala Trp Pro Ala Ala Val Gly Asp Arg Ala Arg Pro Arg Arg Leu	70
780 785 790	
CAG CGC GTG CTG CGC CGC CGG TGC GTC GCG GAG CTG AGC AGG GAG GGG	
Gln Arg Val Leu Arg Arg Cys Val Ala Glu Leu Ser Arg Glu Gly	144
795 800 805	
CCC CAT ATG	
Pro His Met	153
810	
/2) INFORMATION FOR THE PROPERTY OF THE PROPER	
(2) INFORMATION FOR SEQ ID NO:19:	
(i) SEQUENCE CHARACTERISTICS:	
(A) LENGTH: 51 amino acids	
(B) TYPE: amino acid	
(D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: protein	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:	

(B) TYPE: nucleic acid

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	_															
GI	n Ar	g Va	l Le	u Ar	g Ar	g Ar	g Cy	ys Va	l Al	a G	lu Le	eu Se	er Ai	g G]	lu G1	v
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Pr	o Hi	s Me	t													
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(2) IN	FORM	ATIO	N FO	R SE	Q ID	NO:	20:	* .							
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		((C) S	STRA	4DED1	VESS:	do:	uble								
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	(113) HY	POTH	ETIC	:AL:	NO										
	(ix) FE	ATUR	E:												
					vev.	CDS										
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		,	5) L	OCAT	TON:	1	1620)								
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	(xi) SE	QUEN	CE D	ESCR	IPTI	ON:	SEO	ID N	0:20	١.					
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TGC	GTC	GCG	GAG	CTC	200	3.00	a.									
Cvc	W-1	31-	GAG	-	AGC	AGG	GAG	GAC	CTC	GGI	CTC	GAA	CCI	' GAA	GGG	48
cys	vai	Ala	Glu	Leu	Ser	Arg	Glu	Asp	Leu	Gly	Leu	Glu	Pro	Glu	Gly	
			55					60					65			
ATT	GCT	GAA	GGT	TCC	ATC	GAT	AAC	ACA	СТА	GTT	CTC	CCS	3.00		CAA	
Ile	Ala	Glu	Glv	Sor	Tlo	7.00	7	mb	**- 3			GCA	AGT	GAG	CAA	96
		70	- 1	OCL	116	wañ		Inr	vai	val	Val	Ala	Ser	Glu	Gln	
		70					75					80				
GAT	TCT	GAG	ATT	GTG	GTT	GGA	AAG	GAG	CAA	GCT	CGA	GCT	AAA	СТР	ACA	144
Asp	Ser	Glu	Ile	Val	Val	Glv	Lvs	Glii	Gln	Ala	A = ~	31-	T	U-1	Thr	744
	85					90	-1-		O 1.11			urq	råg	val	rnr	
						30					95					
C2.2	3.00															
CAA	AGC	ATT	GTC	TTT	GTA	ACC	GGC	GAA	GCT	TCT	ССТ	TAT	GCA	256	ጥርጥ	192

G1 10	n Se O	r Il	e Va	l Phe	e Va:		Gl;	y Gl	u Al	a Se:		o Tyr	r Ala	a Lys	S Ser	
					Va]					u Pro					GCT Ala	
CG:	r GG	T CAC	C CG: S Arg	y Val	ATC	GTT Val	GTF	A ATO	Pro	C AGA	A TAT	T TTA	Asr	Gly	ACC Thr	288
TC(Ser	GA:	T AAC Lys	G AAT B Asr	TAT	GCA	AAT Asn	Ala	TTI	Ţ TAC	C ACA	A GAP	Lys	His	ATT	CGG Arg	336
	Pro	TGC Cys	TT1					'GAA					CAT		TAT Tyr	384
		TCA		GAC Asp								TCA			AGA	432
180 CCT	GGA	. AAT	TTA	TAT Tyr	185 GGA	GAT	AAG	TTT	GGT	190 GCT	TTT	GGT	GAT	AAT	195 CAG	480
TTC	AGA	TAC	ACA	200 CTC	CTT	TGC	TAT	GCT	205 GCA	TGT	GAG	GCT	CCT	210 TTG	ATC	528
CTT	GAA	TTG	215 GGA	Leu GGA	TAT	ATT	TAT	220 GGA	CAG	AAT	TGC	ATG	225 TTT	GTT	GTC	576
		230		Gly GCC			235					240				624
Asn	Asp 245	Trp	His	Ala	Ser	Leu 250	Val	Pro	Val	Leu	Leu 255	Ala	Ala	Lys	Tyr	624
				GTT Val												672
AAT Asn	TTA Leu	GCA Ala	CAT His	CAG Gln	GGT Gly	GTA Val	GAG Glu	CCT Pro	GCA Ala	AGC Ser	ACA Thr	TAT Tyr	CCT Pro	GAC Asp	CTT Leu	720

	280	289	5 .	290
GGG TTG CCA CC Gly Leu Pro Pro 299	Glu Trp Tyr	GGA GCT CTC Gly Ala Leu 300	G GAG TGG GTA TTC 1 Glu Trp Val Phe 305	Pro Glu
TGG GCG AGG AGG Trp Ala Arg Arg 310	G CAT GCC CTT J His Ala Leu	GAC AAG GGT Asp Lys Gly 315	C GAG GCA GTT AAT Glu Ala Val Asn 320	TTT TTG 816 Phe Leu
AAA GGT GCA GTT Lys Gly Ala Val 325	GGTG ACA GCA Val Thr Ala 330	GAT CGA ATC	GTG ACT GTC AGT Val Thr Val Ser	AAG GGT 864 Lys Gly
TAT TCG TGG GAG Tyr Ser Trp Glu 340	GTC ACA ACT Val Thr Thr 345	GCT GAA GGT Ala Glu Gly	GGA CAG GGC CTC Gly Gln Gly Leu 350	AAT GAG 912 Asn Glu 355
CTC TTA AGC TCC	AGA AAG AGT Arg Lys Ser 360	GTA TTA AAC Val Leu Asn 365	GGA ATT GTA AAT Gly Ile Val Asn	GGA ATT 960
GAC ATT AAT GAT Asp Ile Asn Asp 375	TGG AAC CCT	GCC ACA GAC Ala Thr Asp 380	AAA TGT ATC CCC Lys Cys Ile Pro 385	TGT CAT 1008 Cys His
TAT TCT GTT GAT Tyr Ser Val Asp 390	Asp Leu Ser	GGA AAG GCC Gly Lys Ala 395	AAA TGT AAA GGT Lys Cys Lys Gly 400	GCA TTG 1056 Ala Leu
CAG AAG GAG CTG Gln Lys Glu Leu 405	GGT TTA CCT A	ATA AGG CCT	GAT GTT CCT CTG Asp Val Pro Leu 415	ATT GGC 1104 Ile Gly
TTT ATT GGA AGG Phe Ile Gly Arg 420	TTG GAT TAT C	CAG AAA GGC Gln Lys Gly	ATT GAT CTC ATT Ile Asp Leu Ile 430	CAA CTT 1152 Gln Leu 435
ATC ATA CCA GAT Ile Ile Pro Asp	CTC ATG CGG G Leu Met Arg G 440	AA GAT GTT lu Asp Val 445	Gln Phe Val Met	CTT GGA 1200
TCT GGT GAC CCA Ser Gly Asp Pro 455	GAG CTT GAA G Glu Leu Glu A	AT TGG ATG .sp Trp Met .	AGA TCT ACA GAG	ICG ATC 1248

TT Ph	C AA e Ly	G GA s As	T AAA p Lys	A TT	CG:	r GG	A TG	G GT p Va	T GG l Gl	A TT y Ph	T AG e Se	T GT	T CC	A GT:	TCC Ser	1296
		47	0				47					480				
CA	C CG	A AT	A ACI	GCC	GGG	TG	G GA:	r at	A TT	G TT	A ATO	G CC	A TCC	C AGE	TTC	1344
H1:	48!	g Ile	€ Thr	Ala	Gly	Cys 490	Asy	o Ile	e Le	ı Let	ı Met	t Pro	Ser	Arg	Phe	
											499					
GAZ	A CC	TG	GGT	CTC	AAI	CAC	CTA	A TAT	r GC	TATO	CAC	TAI	GGC	ACA	GTT	1392
500	1 Pro	Cys	Gly	Leu	Asn	Gln	Leu	туг	Ala	a Met	Glr	туг	Gly	Thr	Val	
	•				505					510)				515	
CCI	GTI	GTC	CAT	GCA	ACT	GGG	GGC	CTI	l AGA	GAT	' ACC	GTG	GAG	AAC	TTC	1440
Pro	Val	Val	His	Ala	Thr	Gly	Gly	Leu	Arg	Asp	Thr	Val	Glu	Asn	Phe	1440
				520					525					530		
AAC	CCT	TTC	GGT	GAG	AAT	GGA	GAG	CAG	GGT	ACA	GGG	TGG	GCA	ሞሞር	GCA	1:00
Asn	Pro	Phe	Gly	Glu	Asn	Gly	Glu	Gln	Gly	Thr	Gly	Trp	Ala	Phe	Ala	1488
			535					540					545			
ccc	CTA	ACC	ACA	GAA	AAC	ATG	TTT	GTG	GAC	ΔTT	GCG	ממ	TC C			
Pro	Leu	Thr	Thr	Glu	Asn	Met	Phe	Val	Asp	Ile	Ala	Asn	Cys	AAT	Ile	1536
		550					555					560	•			
TAC	ATA	CAG	GGA	ACA	CAA	GTC	CTC	CTC	CCI	3.00	com					
Tyr	Ile	Gln	Gly	Thr	Gln	Val	Leu	Leu	Gly	Arg	Ala	AAT	GAA	GCG	AGG	1584
	565					570			_	-	575				nry	
CAT	GTC	AAA	AGA	ርጥጥ	ር <u>ነ</u> ር	GTC	CCA	ccs	TC ~	00-	 -					
His	Val	Lys	Arg	Leu	His	Val	Gly	Pro	Cvs	Ara	TGA *					1620
580					585		1		- , 	590						

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 540 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Cys Val Ala Glu Leu Ser Arg Glu Asp Leu Gly Leu Glu Pro Glu Gly

15

- Asp Ser Glu Ile Val Val Gly Lys Glu Gln Ala Arg Ala Lys Val Thr
 35 40 45
- Gln Ser Ile Val Phe Val Thr Gly Glu Ala Ser Pro Tyr Ala Lys Ser 50 55 60
- Gly Gly Leu Gly Asp Val Cys Gly Ser Leu Pro Val Ala Leu Ala Ala 65 70 75 80
- Arg Gly His Arg Val Met Val Val Met Pro Arg Tyr Leu Asn Gly Thr 85 90 95
- Ser Asp Lys Asn Tyr Ala Asn Ala Phe Tyr Thr Glu Lys His Ile Arg
- Ile Pro Cys Phe Gly Gly Glu His Glu Val Thr Phe Phe His Glu Tyr
 115 120 125
- Arg Asp Ser Val Asp Trp Val Phe Val Asp His Pro Ser Tyr His Arg 130 135 140
- Phe Arg Tyr Thr Leu Leu Cys Tyr Ala Ala Cys Glu Ala Pro Leu Ile 165 170 175
- Leu Glu Leu Gly Gly Tyr Ile Tyr Gly Gln Asn Cys Met Phe Val Val 180 185 190
- Asn Asp Trp His Ala Ser Leu Val Pro Val Leu Leu Ala Ala Lys Tyr 195 200 205
- Arg Pro Tyr Gly Val Tyr Lys Asp Ser Arg Ser Ile Leu Val Ile His 210 215 220
- Asn Leu Ala His Gln Gly Val Glu Pro Ala Ser Thr Tyr Pro Asp Leu 225 230 235 240
- Gly Leu Pro Pro Glu Trp Tyr Gly Ala Leu Glu Trp Val Phe Pro Glu

- Trp Ala Arg Arg His Ala Leu Asp Lys Gly Glu Ala Val Asn Phe Leu 260 265 270
- Lys Gly Ala Val Val Thr Ala Asp Arg Ile Val Thr Val Ser Lys Gly 275 280 285
- Tyr Ser Trp Glu Val Thr Thr Ala Glu Gly Gly Gln Gly Leu Asn Glu 290 295 300
- Leu Leu Ser Ser Arg Lys Ser Val Leu Asn Gly Ile Val Asn Gly Ile 305 310 315 320
- Asp Ile Asn Asp Trp Asn Pro Ala Thr Asp Lys Cys Ile Pro Cys His 325 330 335
- Tyr Ser Val Asp Asp Leu Ser Gly Lys Ala Lys Cys Lys Gly Ala Leu 340 345 350
- Gln Lys Glu Leu Gly Leu Pro Ile Arg Pro Asp Val Pro Leu Ile Gly 355 360 365
- Phe Ile Gly Arg Leu Asp Tyr Gln Lys Gly Ile Asp Leu Ile Gln Leu 370 375 380
- Ile Ile Pro Asp Leu Met Arg Glu Asp Val Gln Phe Val Met Leu Gly 385 390 395 400
- Ser Gly Asp Pro Glu Leu Glu Asp Trp Met Arg Ser Thr Glu Ser Ile 405 410 415
- Phe Lys Asp Lys Phe Arg Gly Trp Val Gly Phe Ser Val Pro Val Ser 420 425 430
- His Arg Ile Thr Ala Gly Cys Asp Ile Leu Leu Met Pro Ser Arg Phe 435 440 445
- Glu Pro Cys Gly Leu Asn Gln Leu Tyr Ala Met Gln Tyr Gly Thr Val 450 455 460
- Pro Val Val His Ala Thr Gly Gly Leu Arg Asp Thr Val Glu Asn Phe 465 470 475 480
- Asn Pro Phe Gly Glu Asn Gly Glu Gln Gly Thr Gly Trp Ala Phe Ala

1 85	490	495
		447

Pro Leu Thr Thr Glu Asn Met Phe Val Asp Ile Ala Asn Cys Asn Ile 500 505 510

Tyr Ile Gln Gly Thr Gln Val Leu Leu Gly Arg Ala Asn Glu Ala Arg

His Val Lys Arg Leu His Val Gly Pro Cys Arg * 530 535 540

- (2) INFORMATION FOR SEQ ID NO:22:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "Oligonucleotide"
 - (iii) HYPOTHETICAL: NO
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GTGGATCCAT GGCGACGCCC TCGGCCGTGG

- (2) INFORMATION FOR SEQ ID NO:23:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 35 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "Oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:	
CTGAATTCCA TATGGGGCCC CTCCCTGCTC AGCTC	35
(2) INFORMATION FOR SEQ ID NO:24:	
(i) SEQUENCE CHARACTERISTICS:	
(A) LENGTH: 36 base pairs	
(B) TYPE: nucleic acid	
(C) STRANDEDNESS: single	
(D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: other nucleic acid	
(A) DESCRIPTION: /desc = "Oligonucleotide"	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:	
CTCTGAGCTC AAGCTTGCTA CTTTCTTTCC TTAATG	36
(2) INFORMATION FOR SEQ ID NO:25:	
(i) SEQUENCE CHARACTERISTICS:	
(A) LENGTH: 29 base pairs	
(B) TYPE: nucleic acid	
(C) STRANDEDNESS: single	
(D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: other nucleic acid	
(A) DESCRIPTION: /desc = "Oligonucleotide"	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:	
GTCTCCGCGG TGGTGTCCTT GCTTCCTAG	29
(2) INFORMATION FOR SEQ ID NO:26:	
(i) SEQUENCE CHARACTERISTICS:	
(A) LENGTH: 53 base pairs	

(B) TYPE: nucleic acid

- (C) STRANDEDNESS: double
- (D) TOPOLOGY: not relevant
- (ii) MOLECULE TYPE: cDNA to mRNA
- (iii) HYPOTHETICAL: NO
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

TGCGTCGCGG AGCTGAGCAG GGAGGTCTCC GCGGTGGTGT CCTTGCTTCC TAG

53

- (2) INFORMATION FOR SEQ ID NO:27:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 8 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: not relevant
 - (ii) MOLECULE TYPE: peptide
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Cys Val Ala Glu Leu Ser Arg Glu 1 5

- (2) INFORMATION FOR SEQ ID NO:28:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 16 base pairs
 - (B) TYPE: nucleic acid
 - (.C) STRANDEDNESS: double
 - (D) TOPOLOGY: not relevant
 - (ii) MOLECULE TYPE: cDNA to mRNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:	
AGAGAGAG AGAGAG	16
(2) INFORMATION FOR SEQ ID NO:29:	
(i) SEQUENCE CHARACTERISTICS:	
(A) LENGTH: 36 base pairs	
(B) TYPE: nucleic acid	
(C) STRANDEDNESS: double	
(D) TOPOLOGY: not relevant	
(ii) MOLECULE TYPE: cDNA to mRNA	
(iii) HYPOTHETICAL: NO	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:	
AAGAAGAAGA AGAAGAAGAAG AAGAAG	36
(2) INFORMATION FOR SEQ ID NO:30:	
(i) SEQUENCE CHARACTERISTICS:	
(A) LENGTH: 18 base pairs	
(B) TYPE: nucleic acid	
(C) STRANDEDNESS: double	
(D) TOPOLOGY: not relevant	
(ii) MOLECULE TYPE: cDNA to mRNA	
(iii) HYPOTHETICAL: NO	
	•
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:	
AAAAAAA AAAAAAA	18
(2) INFORMATION FOR SEQ ID NO:31:	
(i) SEQUENCE CHARACTERISTICS:	

(C) STRANDEDNESS: single (D) TOPOLOGY: not relevant (ii) MOLECULE TYPE: other nucleic acid (A) DESCRIPTION: /desc = "Oligonucleotide" (iii) HYPOTHETICAL: NO (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31: AGATAATGCA G 11 (2) INFORMATION FOR SEQ ID NO: 32: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 10 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: not relevant (ii) MOLECULE TYPE: other nucleic acid (A) DESCRIPTION: /desc = "Oligonucleotide" (iii) HYPOTHETICAL: NO (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32: AACAATGGCT 10 (2) INFORMATION FOR SEQ ID NO:33: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 56 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: not relevant

(A) LENGTH: 11 base pairs(B) TYPE: nucleic acid

- (ii) MOLECULE TYPE: peptide (iii) HYPOTHETICAL: NO... (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33: Met Ala Ser Ser Met Leu Ser Ser Ala Ala Val Ala Thr Arg Thr Asn 5 10 15 Pro Ala Gln Ala Ser Met Val Ala Pro Phe Thr Gly Leu Lys Ser Ala 20 25 Ala Phe Pro Val Ser Arg Lys Gln Asn Leu Asp Ile Thr Ser Ile Ala 35 40 Ser Asn Gly Gly Arg Val Gln Cys 50 55 (2) INFORMATION FOR SEQ ID NO:34: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 58 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: not relevant (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

(iii) HYPOTHETICAL: NO

Met Ala Pro Thr Val Met Met Ala Ser Ser Ala Thr Ala Thr Arg Thr 1 5 10 15

Asn Pro Ala Gln Ala Ser Ala Val Ala Pro Phe Gln Gly Leu Lys Ser

Thr Ala Ser Leu Pro Val Ala Arg Arg Ser Ser Arg Ser Leu Gly Asn

Val Ala Ser Asn Gly Gly Arg Ile Arg Cys 50 55

- (2) INFORMATION FOR SEQ ID NO:35:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 58 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: not relevant
 - (ii) MOLECULE TYPE: peptide
 - (iii) HYPOTHETICAL: NO
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Met Ala Gln Ile Leu Ala Pro Ser Thr Gln Trp Gln Met Arg Ile Thr 1 5 10 15

Lys Thr Ser Pro Cys Ala Thr Pro Ile Thr Ser Lys Met Trp Ser Ser 20 25 30

Leu Val Met Lys Gln Thr Lys Lys Val Ala His Ser Ala Lys Phe Arg 35 40 45

Val Met Ala Val Asn Ser Glu Asn Gly Thr 50 55

- (2) INFORMATION FOR SEQ ID NO:36:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 74 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: not relevant
 - (ii) MOLECULE TYPE: peptide
 - (iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Met Ala Ala Leu Ala Thr Ser Gln Leu Val Ala Thr Arg Ala Gly His 1 5 10 15

Gly Val Pro Asp Ala Ser Thr Phe Arg Arg Gly Ala Ala Gln Gly Leu 20 25 30

Arg Gly Ala Arg Ala Ser Ala Ala Ala Asp Thr Leu Ser Met Arg Thr 35 46 45

Ser Ala Arg Ala Ala Pro Arg His Gln Gln Gln Ala Arg Arg Gly Gly 50 55 60

Arg Phe Pro Phe Pro Ser Leu Val Val Cys 65 . 70

- (2) INFORMATION FOR SEQ ID NO:37:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 39 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: not relevant
 - (ii) MOLECULE TYPE: peptide
 - (iii) HYPOTHETICAL: NO
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Met Ala Thr Pro Ser Ala Val Gly Ala Ala Cys Leu Leu Leu Ala Arg 1 5 10 15

Xaa Ala Trp Pro Ala Ala Val Gly Asp Arg Ala Arg Pro Arg Arg Leu 20 25 30

Gln Arg Val Leu Arg Arg Arg 35